

Katja Hätönen

# Challenges in Measuring Glycaemic Index





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Katja Hätönen

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## **ACADEMIC DISSERTATION**

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Department of Lifestyle and Participation,  
National Institute for Health and Welfare, Helsinki, Finland  
and  
Department of Chronic Disease Prevention,  
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and  
Department of Food and Environmental Science,  
Faculty of Agriculture and Forestry, University of Helsinki

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**Supervisors**

Adjunct Professor Liisa Valsta, PhD  
Department of Lifestyle and Participation  
National Institute for Health and Welfare  
Helsinki, Finland (on leave of absence)  
and Evidence Management Unit  
European Food Safety Authority (EFSA)  
Parma, Italy

and Research Professor Emeritus Jarmo Virtamo, MD, PhD  
Department of Chronic Disease Prevention  
National Institute for Health and Welfare  
Helsinki, Finland

and Professor Johan Eriksson, MD, DMSc  
Department of General Practice and Primary Health Care  
University of Helsinki  
Helsinki, Finland  
and Department of Chronic Disease Prevention  
National Institute for Health and Welfare  
Helsinki, Finland

**Reviewers**

Professor Anne Raben, PhD  
Department of Nutrition, Exercise and Sports  
University of Copenhagen  
Copenhagen, Denmark

and Adjunct Professor Marjukka Kolehmainen, PhD  
Department of Clinical Nutrition  
Institute of Public Health and Clinical Nutrition  
University of Eastern Finland  
Kuopio, Finland

**Opponent**

Academy Professor Kaisa Poutanen, DTech  
Technical Research Centre of Finland (VTT)  
Espoo, Finland  
and Department of Clinical Nutrition  
Institute of Public Health and Clinical Nutrition  
University of Eastern Finland  
Kuopio, Finland



To my Family





## Abstract

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Glycaemic response to carbohydrate-containing foods is a combination of glucose absorption, endogenous glucose production, and tissue glucose uptake. After a carbohydrate-containing meal, blood glucose rises, which stimulates insulin secretion. The different amount and type of carbohydrates influence postprandial glucose and insulin responses differently. The concept of the glycaemic index (GI) was developed to rank carbohydrates according to their effect on blood glucose levels. Food with a low GI value is considered beneficial in maintaining optimal blood glucose due to smaller incremental increase in blood glucose than food with a high GI value. Foods characterized by a low GI therefore have been found to induce benefits on several risk markers related to type 2 diabetes and cardiovascular disease. The concept of the GI was originally developed as a tool for individuals with diabetes in choosing the most beneficial carbohydrate-rich foods regarding blood glucose responses. To assess the extent to which eaten food raises insulin levels, the concept of the insulinaemic index (II) was launched. The calculation of II values is performed similarly to GI values.

The concept of GI has been widely studied and debated in the scientific literature. The main aim of this thesis was to investigate the effect of methodological choices on measured glucose and insulin responses, especially on GI values. To achieve these goals, five different postprandial studies were conducted in healthy individuals and in individuals with impaired glucose tolerance. Capillary and venous blood samples were collected up to 2-3 h postprandially and the incremental area under the curve (IAUC), GIs, and IIs were calculated.

In the first study, the effects of methodological choices on measured glycaemic response and GI values were determined. Comparisons were done between blood sampling type (capillary vs. venous blood samples), type of reference food (white bread vs. glucose solution), and how many times the reference food should be repeated. Results revealed that the variation was smaller when capillary samples were used, performing the reference food test twice is sufficient, and to achieve better accuracy the glucose solution should be used as the reference food.

In the second study, the effects of coffee on postprandial glucose and insulin responses were determined. Coffee as such does not modify the glucose and insulin responses of a carbohydrate food. Coffee had no marked effect on GI values.

In the third study, the effects of subjects' physiological characteristics, namely glucose tolerance and overweight, on postprandial glucose and insulin responses were examined. Both overweight and impaired glucose tolerance increased glycaemic and insulinaemic responses to the tested meals and the reference food. As a consequence, physiological characteristics did not affect the measured GI values.

In the fourth study, the effects of other macronutrients, namely fat and protein, on glycaemic responses to a starchy food were examined. Both fat and protein have an independent decreasing effect on glycaemia, and as a consequence, GI values

diminished. Insulin responses to the meals were also measured. Adding protein to the mashed potato-based meals considerably enhanced insulin responses to the meal.

In the fifth study, the effects of alcohol on postprandial glucose and insulin responses were determined. Alcohol was found to increase postprandial glucose and insulin responses, probably through acutely increased insulin resistance. In addition, high GI values were measured for both beer and non-alcoholic beer. This should be taken into account when GI databases are compiled for epidemiologic studies.

In summary, several factors affect measured GI values, highlighting that different methodological choices should be carefully considered. The use of recent international standards, for measuring GI values is highly recommended, and GI values measured prior to the standard should be interpreted and utilized with caution. To increase the reliability of GI measurements in the future, GI should be measured in combination with II measurements.

**Keywords:** Glycaemic index, insulinaemic index, glucose response, insulin response, postprandial responses

## Tiivistelmä

Katja Hätönen. Challenges in Measuring Glycemic Index [Glykeemisen indeksin mittaamisen haasteet]. Terveyden ja hyvinvoinnin laitos (THL). Tutkimus 135. 150 sivua. Helsinki, Finland 2014. ISBN 978-952-302-289-8 (printed); ISBN 978-952-302-290-4 (pdf)

Hiilihydraattipitoisen elintarvikkeen verensokerivasteen muodostumiseen vaikuttavat hiilihydraattien imeytyminen, kehon oma glukoosintuotanto ja kudosten glukoosin sisäänotto. Aterian jälkeen verensokeri nousee, mikä stimuloi insuliinin eritystä. Erilaiset hiilihydraatit vaikuttavat aterianjälkeisiin verensokeri(glukoosi)- ja insuliinivasteisiin eri tavoin. Glykeeminen indeksi (GI) kehitettiin luokittelemaan hiilihydraattipitoisia elintarvikkeita niiden aikaansaamien verensokerivasteiden perustella. Elintarvikkeita, joilla on pieni GI, pidetään hyödyllisenä optimaalisen verensokeritason kannalta. Pienen GI:n elintarvikkeet nostavat verensokeria maltillisesti verrattuna suuren GI:n elintarvikkeisiin. Tästä johtuen pienen GI:n elintarvikkeiden syömisestä on osoitettu olevan hyötyä useiden kroonisten tautien, kuten tyypin 2 diabeteksen ja sydän- ja verisuonitautien, liittyvien riskitekijöiden kannalta. GI:n konsepti luotiin alun perin diabeetikoiden avuksi, jotta he voisivat valita mahdollisimman edullisia elintarvikkeita verensokerivasteiden kannalta. Syödyn elintarvikkeen insuliinivasteiden luokittelua varten kehitettiin insulineeminen indeksi (II), joka mitataan ja lasketaan samalla tavoin kuin elintarvikkeen GI.

GI-konsepti on paljon tutkittu ja tieteellinen keskustelu aiheesta on ollut runsasta. Tämän väitöstutkimuksen tarkoituksena oli tutkia menetelmällisten valintojen vaikutusta mitattuihin glukoosi- ja insuliinivasteisiin sekä etenkin GI-arvoihin. Väitöstutkimusta varten tehtiin viisi erillistä koesarjaa, joista neljä toteutettiin terveillä tutkittavilla ja yhdessä koesarjassa oli terveiden tutkittavien lisäksi tutkittavia, joiden glukoosinsieto oli heikentynyt. Tutkittavilta otettiin sormenpäästä ja laskimoista verinäytteitä kahden tai kolmen tunnin ajan. Näiden säännöllisin väliajoin otettujen verinäytteiden avulla määritettiin verensokeri- ja insuliinivasteet sekä laskettiin GI- ja II-arvot.

Ensimmäisessä koesarjassa tutkittiin menetelmällisten valintojen vaikutusta mitattuihin verensokerivasteisiin ja GI-arvoihin. Vertailuja tehtiin sormenpää- ja laskimoverinäytteiden välillä, kahden erilaisen vertailuelintarvikkeen (vaalea leipä vs. glukoosiliuos) ja sen välillä, kuinka monta kertaa vertailuelintarvike pitää testata. Tulokset osoittivat vaihtelun olevan pienempää, kun sormenpääverinäytteitä käytettiin verensokerivasteiden ja GI-arvojen mittaamiseen. Tulosten perusteella voidaan todeta, että vertailuelintarvikkeen toistaminen kerran aikaansaa riittävän mittaustarkkuuden ja vertailuelintarvikkeena tulisi käyttää glukoosiliuosta.

Toisessa koesarjassa tutkittiin vaikuttaako kahvi aterianjälkeisiin verensokeri- ja insuliinivasteisiin. Kahvi ei muokannut hiilihydraattipitoisen elintarvikkeen verensokeri- ja insuliinivasteita. Tästä johtuen kahvi ei vaikuta GI-arvojen mittaamiseen.

Kolmannessa koesarjassa tutkittiin tutkittavien fysiologisten ominaisuuksien, kuten glukoosinsiedon ja ylipainon, vaikutusta aterianjälkeisiin verensokeri- ja

insuliinivasteisiin. Havaittiin, että tutkittavan ylipaino ja heikentynyt sokerinsieto suurentavat verensokeri- ja insuliinivasteita. Ylipainolla ja heikentyneellä sokerinsiedolla ei kuitenkaan havaittu olevan vaikutusta GI-arvoihin.

Neljännessä koesarjassa tutkittiin energiaravintoaineiden, rasvan ja proteiinin, vaikutusta tärkkelyspitoisen elintarvikkeen aikaansaamiin verensokerivasteisiin. Sekä rasva että proteiini pienensivät aterian aikaansaamaa verensokerivastetta. Tämän seurauksena GI-arvot laskivat. Koesarjassa määritettiin myös aterioiden insuliinivaste. Proteiinin lisääminen perunamuusiateriaan kasvatti insuliinivasteita huomattavasti.

Viidennessä koesarjassa tutkittiin alkoholin vaikutusta verensokeri- ja insuliinivasteisiin. Tutkimuksessa havaittiin, että alkoholi suurentaa verensokeri- ja insuliinivasteita. Tämä vaikutus välittyi todennäköisesti huonontuneen insuliiniherkkyyden kautta. Koesarjassa mitattiin suuret GI-arvot oluelle ja alkoholittomalle oluelle. Tämä havainto pitäisi ottaa huomioon, kun koostetaan GI-tietokantoja epidemiologisia tutkimuksia varten.

Yhteenvetona voidaan todeta useiden tekijöiden vaikuttavan mitattuihin GI-arvoihin. Tästä johtuen metodologisiin valintoihin tulee erityisesti kiinnittää huomiota. Määritettäessä GI-arvoja tulee noudattaa äskettäin julkaistua kansainvälistä standardia ja aiemmin mitattuja GI-arvoja pitää tulkita ja käyttää varoen. Jatkossa II-arvot tulisi mitata samanaikaisesti GI-arvojen kanssa. Käytäntö lisääisi mitattujen GI-arvojen luottavuutta.

Avainsanat: Glykeeminen indeksi, insulineeminen indeksi, glukoosivaste, insuliinivaste, aterianjälkeiset vasteet

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## List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

- I Hätönen KA, Similä ME, Virtamo JR, Eriksson JG, Hannila M-L, Sinkko HK, Sundvall JE, Mykkänen HM, Valsta LM. Methodologic considerations in the measurement of glycemic index: glycemic response to rye bread, oatmeal porridge, and mashed potato. *Am J Clin Nutr* 2006;84:1055-61.
- II Hätönen KA, Virtamo J, Eriksson JG, Sinkko HK, Erlund I, Jousilahti P, Leiviskä JM, Valsta LM. Coffee does not modify postprandial glycaemic and insulinaemic responses induced by carbohydrates. *Eur J Nutr* 2012;51:801-806.
- III Perälä MM, Hätönen KA, Virtamo J, Eriksson JG, Sinkko HK, Sundvall J, Valsta LM. Impact of overweight and glucose tolerance on postprandial responses to high and low glycaemic index meals. *Br J Nutr* 2011;105:1627-1634.
- IV Hätönen KA, Virtamo J, Eriksson JG, Sinkko H, Sundvall J, Valsta LM. Protein and fat modify the glycaemic and insulinaemic responses of mashed potato-based meal. *Br J Nutr* 2011;106:248-253.
- V Hätönen KA, Virtamo J, Eriksson JG, Perälä M-M, Sinkko HK, Leiviskä J, Valsta LM. Modifying effects of alcohol on the postprandial glucose and insulin responses in healthy subjects. *Am J Clin Nutr* 2012;96:44-49.

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## Abbreviations

ACHO	Available Carbohydrates
ANOVA	Analysis of Variance
AUC	Area under the Curve
BMI	Body Mass Index
CCK	Cholecystokinin
CV	Coefficient of Variation
CVD	Cardiovascular Disease
FAO	Food and Agricultural Organization
FFQ	Food Frequency Questionnaire
GI	Glycaemic Index
GIP	Gastric Inhibitory Peptide
GLP-1	Glucagon-Like Peptide 1
HOMA1-IR	Homeostasis Model Assessment of Insulin Resistance
IAUC	Incremental Area under the Curve
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
II	Insulinaemic Index
MMTT	Mixed-meal Tolerance Test
NGT	Normal Glucose Tolerance
OGTT	Oral Glucose Tolerance Test
PUFA	Polyunsaturated Fatty Acid



PYY	Peptide YY
RCT	Randomized Controlled Trial
RGR	Relative Glycaemic Response
SCFA	Short-chain Fatty Acid
SD	Standard Deviation
SEM	Standard Error of Mean
SFA	Saturated Fatty Acid
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
WHO	World Health Organization



# 1 Introduction

The quality of carbohydrates has been heavily debated in recent years. A joint FAO/WHO committee has recommended the consumption of a diet containing at least 55% of energy from carbohydrates to maintain health and prevent diseases (FAO/WHO 1998). Similar nutritional recommendations regarding the amount of carbohydrate and dietary fibre exist in Europe and America (EFSA 2010, Dietary Guidelines for Americans 2010). Interest in different carbohydrates is not a new issue. Already at the beginning of the 20<sup>th</sup> century, studies were conducted regarding the potency of carbohydrate-rich foods to increase blood glucose (Conn and Newburgh 1936). Glycaemic responses to various carbohydrate-containing foods were investigated more comprehensively in the 1970s (Otto and Niklas 1980). Later, the concept of the glycaemic index (GI) was introduced as an alternative system for classifying carbohydrate-containing foods (Jenkins et al. 1981). More recently, also insulin responses to foods were measured, and, correspondingly to the GI value, insulinaemic indices (IIs) were calculated (Bornet et al. 1987, Holt et al. 1992).

The nutritional properties of carbohydrates are influenced by the rate and extent of digestion and absorption in the small intestine (Wong and Jenkins 2007). Several factors affect postprandial glucose and insulin responses of carbohydrates and carbohydrate-containing foods, e.g. the chemical structure of a carbohydrate, food processing, and storage condition (Brand et al. 1985, Burton and Lightowler 2008, Larsen et al. 2000, Simpson et al. 1985b). In addition, other components of the ingested food, e.g. organic acids, fibre, protein, and fat, modify postprandial responses (Björck et al. 1994, Nuttall et al. 1984). There are also data on a second-meal effect indicating that the quality of a preceding meal impacts the glucose responses to the following meal (Axelsen et al. 1999, Axelsen et al. 2000, Granfeldt et al. 2006, Wolever et al. 1988c). These factors potentially provide a source of variability in both GIs and the day-to-day variability of glycaemic responses to the same food (Venn and Green 2007). However, the most important factor determining postprandial glucose and insulin responses is the amount of available carbohydrates food consumed (Wolever and Bolognesi 1996b, Wolever 2000). It is obvious that modulating carbohydrate digestion patterns can affect health in many ways. Postprandial hyperglycaemia and compensatory hyperinsulinaemia have been linked to the development of lifestyle-related chronic diseases such as type 2 diabetes and coronary heart disease (Giugliano et al. 2008, Riccardi et al. 2008). The risk of chronic diseases can be potentially modified by reducing glycaemic response to foods. It is supposed that low-GI foods cause a reduced rate of glucose absorption, which, in turn, elicits a diminished postprandial insulin response (Björck et al. 2000, Ludwig 2002). However, this assumption is not widely confirmed, with, for instance, inconsistencies occurring between glucose and insulin responses to milk

products (Östman et al. 2001). Protein increases the insulin response to carbohydrate-containing foods (Bao et al. 2011, Holt et al. 1997).

Despite three decades of research, the GI remains a contentious issue. Numerous GI values have been published to date. In the original study launching the GI concept, GI values for 62 food items were listed (Jenkins et al. 1981). The first international GI database was published in 1995 (Foster-Powell and Miller 1995). GI data have been compiled over time from different laboratories. However, the methodological choices have varied markedly between different laboratories, influencing the GIs measured. Since 1998, there has been an internationally accepted protocol for measuring GI values, but the protocol does not address all common methodological variations (FAO/WHO 1998). Vast variation exists in GI values of some food items listed in the international GI tables, which suggests that some GI testing groups are not using or only partially adhere to the WHO protocol (Nordic Council of Ministers. 2005).

This thesis aims to evaluate the effects of different methodological choices, e.g. type of blood sampling, choice of reference food, and number of the test of a reference food is repeated, on GI testing, the effect of subjects' physiological background on GI values, and furthermore, the effects of macronutrients, fat, protein, and alcohol on postprandial glycaemia.

## 2 Review of the literature

Carbohydrates are macronutrients that yield energy. The predominant carbohydrate-containing food groups in human nutrition are cereals, sweeteners, root crops, pulses, vegetables, fruit, and milk products. Of these, cereals are worldwide the most important source of energy in human nutrition (FAO/WHO 1998).

Carbohydrates can be classified based on the degree of polymerization. Carbohydrates are divided into three principal groups, i.e. sugars, oligosaccharides, and polysaccharides. The group of sugars consists of monosaccharides, e.g. glucose, fructose, and galactose, disaccharides, e.g. sucrose and lactose, and polyols (sugar alcohols), e.g. xylitol and sorbitol. The group of oligosaccharides consists of malto-oligosaccharides, raffinose, and fructo-oligosaccharides. The group of polysaccharides can be divided into starch and dietary fibre (non-starch polysaccharides). Carbohydrates can be also classified according to their physiological properties. Carbohydrates are then categorized as glycaemic and non-glycaemic carbohydrates, which simply means that glycaemic carbohydrates are capable of increasing blood glucose concentration, and non-glycaemic are not. Sugars, oligosaccharides, and starch are glycaemic carbohydrates, and dietary fibre and resistant starch are non-glycaemic carbohydrates (Cummings and Stephen 2007).

### 2.1 Glycaemic index (GI), insulinaemic index (II), and glycaemic load (GL)

Available carbohydrates (ACHO) are glycaemic carbohydrates, i.e. they increase blood glucose concentration, which in turn stimulates insulin secretion. Concentrations of blood glucose and insulin are primarily determined by intake of dietary carbohydrates, but such factors as body weight, genetic background, and epigenetic factors also have an effect. The concept of glycaemic index (GI) was introduced to classify the different sources of carbohydrates (CHO) and carbohydrate-rich foods according to their postprandial glycaemic responses (Jenkins et al. 1981). In other words, it is a method to physiologically classify carbohydrate-containing foods. GI was originally proposed for foods providing over 80% of their energy from available carbohydrates (Brouns et al. 2005). GI is defined as the incremental area under the blood glucose response curve (IAUC) of a 50-g carbohydrate portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a reference food consumed by the same subject (FAO/WHO 1998). The GI of a reference food is defined as 100. The GI values of foods are arbitrarily classified into three categories: a GI value of 55 or less is

considered low, 56-69 as medium, and 70 or more as high (International Standard ISO 26642:2010. 2010).

Insulin secretion is mainly stimulated by carbohydrates. However, postprandial insulin responses are not constantly proportional to blood glucose levels or to the carbohydrate content of the meal. Several insulinotropic factors are also recognized, including fructose, certain amino acids and fatty acids, and gastrointestinal hormones such as glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), and cholecystokinin (Collier et al. 1988). Initially, postprandial insulin responses were determined because it was suggested that merely measuring glycaemic response is insufficient (Coulston et al. 1984b, Hollenbeck et al. 1986). As a consequence, insulinaemic indices (IIs) were begun to be measured for carbohydrate-rich foods (Bornet et al. 1987, Brand Miller et al. 1995, Holt et al. 1992). The II indicates the insulinaemic response to different carbohydrate sources. II is measured and calculated in a similar way as GI values (WHO/FAO 1998). However, for II values no categories classifying values from low to high exist.

A considerable limitation of GI is that it is only a qualitative measure of carbohydrate that focuses on the ability of a carbohydrate to raise blood glucose. It does not take into account the effect of carbohydrate portion size on postprandial glucose responses. Thus, the concept of glycaemic load (GL) was introduced to account for the quantity of carbohydrate consumed. GL is proposed to be a better predictor of postprandial glucose response and insulin demand than available carbohydrate alone (Bao et al. 2011). GL is defined as the mathematical product of the GI of a food and its carbohydrate content (g) divided by 100 ( $GL = GI/100 \times \text{amount of available carbohydrate}$ ) (Salmeron et al. 1997). By definition, foods can be classified as having low ( $\leq 10$ ), medium ( $10 < 20$ ), or high ( $\geq 20$ ) GL value (Venn and Green 2007).

## 2.2 Protocol for measurement of GI

After launching the concept of GI, several studies have used various procedures for measuring GI values. Typically, the protocol used has been adapted from the original procedure described by Wolever *et al.* (1991), which is in line with the protocol recommended by the FAO/WHO. The FAO/WHO expert report, published in 1998, has been referred to as the international standard. The protocol of determining GI values based on the recommendation of the FAO/WHO (1998) is briefly summarized as follows:

- At least six subjects should be studied
- The portion of the study meal (the test or the reference food) should contain 50 g of available carbohydrate, and it should be given to the participant after a 10- to 12-h overnight fast
- The study meals should be tested in random order on separate days
- A standard beverage of water, tea, or coffee should be given with each study meal
- The reference food can be either white bread or a glucose solution, and the reference food should be tested at least three times in each subject
- Either capillary or venous blood sampling can be used

Recent recommendations suggest that the amount of available carbohydrate be 25 g with foods having a low carbohydrate density to avoid a large test meal size (Brouns et al. 2005). The recommendation of the FAO/WHO (1998) also demonstrates how the GI can be applied to mixed meals or diets by calculating the weighted GI value of a meal or diet.

## 2.3 GI and GL and chronic diseases

The concept of GI was originally developed to guide diabetic patients in food selection, with the focus on selecting foods with a low GI value. The underlying principle is that carbohydrates with a low GI value are absorbed at a slower rate, leading to a lower rise in blood glucose level (Brand et al. 1991). High-GI meals produce an initial period of high blood glucose and insulin concentrations, followed by reactive hypoglycaemia, counter-regulatory hormone secretion, and elevated nonesterified fatty acids (NEFAs) (Ludwig 2002). Evidence also suggests that diets with high GI or GL can cause oxidative stress and inflammatory responses (Kristo et al. 2013, Levitan et al. 2008).

A consensus exists that a low or attenuated glycaemic response is beneficial for both healthy and diabetic persons. Low-GI foods are useful in management of diabetes, obesity, and cardiovascular diseases (CVDs) (Ajala et al. 2013, Kelly et al. 2004, Thomas et al. 2007, Thomas and Elliott 2009). A recent randomized controlled trial did not, however, find a difference in weight loss when a moderate-carbohydrate (energy from carbohydrates 42%) low-GI diet was compared with a moderate-carbohydrate high-GI diet (Juanola-Falgarona et al. 2014). An earlier result was that a modest reduction in diet GI led to maintenance of weight loss after

a 26-week period (Larsen et al. 2010). However, a sustained effect of lower diet GI on weight regain was not observed in the subgroup analysis after one year (Aller et al. 2014).

Meta-analyses of prospective cohort studies have shown that dietary GI and GL are positively associated with increased risk of type 2 diabetes in both genders (Bhupathiraju et al. 2014, Livesey et al. 2013) and with coronary heart disease (CHD) events in women, but not in men (Mirrahimi et al. 2012). According to a meta-analysis of randomized controlled trials, low-GI meals improved blood glucose control in people with diabetes (Brand-Miller et al. 2003). In addition, a recent meta-analysis of RCTs has revealed a beneficial effect of long-term low-GI diets on fasting insulin in overweight subjects (Schwingshackl and Hoffmann 2013).

An increased consumption of low-GI foods and substituting low-GL foods for higher GL foods are advocated in nutritional recommendations (Evert et al. 2014, FAO/WHO 1998). Some concerns exist about nutrient adequacy when low-GI diets are followed, especially if nutritious high-GI foods, such as whole-grain breads and potatoes, are excluded from the diet. A recent study revealed, however, that a low-GI diet can be a more nutritionally adequate diet than a high-GI diet (Louie et al. 2012). Nevertheless, excluding high-GI whole-grain breads may not be wise because whole-grain breads, especially rye breads, have been shown to have positive effects on insulin metabolism (Juntunen et al. 2003b, Laaksonen et al. 2005).

## 2.4 Methodological choices affecting GI values

Several factors, including type of starch, fibre content, ripeness, fat content, acid content, polyphenol content, and the physical form of an eaten food, can affect glycaemic response to the food (Aldughpassi et al. 2012, Björck et al. 1994, Törrönen et al. 2013). In general, it is incorrect to assume that all simple sugars (monosaccharides and disaccharides) have high GI values and “complex” carbohydrates, such as whole-grains or high-fibre foods, have low GI values. GI values for the major dietary sugars vary between 20 for fructose and 108 for maltose (Atkinson et al. 2008). Sucrose has a medium GI of ~60 because it contains only half the glucose-equivalents of an equal amount of glucose or starch. Intake of high-GI foods, including sugar-sweetened beverages (irrespective of GI value), results in a rapid rise in blood glucose, whereas low-GI foods, including non-starchy vegetables, milk, most fruits, legumes, and nuts, digest slower, therefore resulting in a more gradual rise in blood sugar levels (Brand-Miller et al. 2009). Accordingly, GI values do not directly correlate to the molecular weight of the carbohydrate component *per se* (Björck et al. 2000). For example, monosaccharides, glucose, and fructose have very different GI values (Jenkins et al. 1981, Lee and Wolever 1998). Differences in GI values are explained by the metabolism of glucose and fructose.



Firstly, fructose is poorly absorbed, and secondly, it has to be converted to glucose in the liver (Riby et al. 1993, Sun and Empie 2012).

In the Western world, carbohydrate-containing staple foods, including breads, potatoes, breakfast cereals, porridges, and other processed cereal foods, typically have high GI values due to the high degree of starch gelatinization, which leads to more rapid digestion and absorption (Atkinson et al. 2008). Current processing methods allow the starch to become fully hydrated and, as a consequence, rapidly hydrolysed into glucose in the intestinal tract, which may lead to high GI values among most varieties of potato products, bread, breakfast cereals, and porridges (Brand et al. 1985). Even storage and preparation conditions, namely freezing and toasting, have an influence on glycaemic response to white bread and may affect GI values. A recent study reported that frozen and toasted white bread produced significantly lower (up to ~46% lower depending on condition) glycaemic responses than fresh white bread. The breads were tested in equivalent amounts of available carbohydrate (Burton and Lightowler 2008). Furthermore, reducing white bread volume from 3000 ml to 2400, 1700, and 1100 ml led to 14%, 28% and 62% reductions in GI values, respectively. All tested breads contained 50 g of available carbohydrate (Burton and Lightowler 2006).

Most information regarding starchy foods indicates a linear correlation between GIs and IIs, suggesting that low-GI foods are also less insulin-demanding (Björck et al. 2000). On the other hand, glucose responses are not always proportional to insulin responses (Holt et al. 1997). Inconsistency between glucose and insulin responses to milk products (typically low GI, but high II) and to rye products (typically high glucose responses, but moderate insulin responses) has been found in both healthy subjects and subjects with type 2 diabetes (Gannon et al. 1986, Juntunen et al. 2003a, Östman et al. 2001).

#### 2.4.1 Method of blood sampling

According to the FAO/WHO protocol, both capillary and venous blood sampling are considered acceptable to assess glycaemic responses to food. Both sampling methods are widely used (Atkinson et al. 2008), but the recent recommendation favours capillary blood sampling (Brouns et al. 2005). Differences between glycaemic responses may potentially also be due to the method of blood sampling. In postprandial studies, capillary blood sampling has produced a greater magnitude and less variability in glucose response than venous blood sampling (Granfeldt et al. 1995, Wolever et al. 1988b, Wolever and Bolognesi 1996a, Wolever et al. 2003, Vrolix and Mensink 2010). This suggests that capillary blood sampling increases sensitivity in GI testing (Wolever et al. 2003).

The FAO/WHO recommends drawing seven blood samples over 2 h when determining GI. The blood samples should be taken at 15-min intervals during the

first hour and at 30-min intervals during the second hour after the study meal. The area under the curve (AUC) should be calculated as the incremental AUC, ignoring the area beneath the fasting concentration (FAO/WHO 1998). The method was firstly described in detail by Wolever and Jenkins (1986). However, alternative methods of calculating AUC have also been used (Ha et al. 1992, Wolever 1989, Wolever 2004).

If the area below the fasting concentration was included in the calculation of the AUC, the AUC would be larger than ignoring the area below the fasting concentration. This means that GI values, as a consequence, may be smaller. However, the greater problem is the presence of a correlation between the GI value and the AUC. This may indicate that the method is invalid because the fasting glucose and the glucose tolerance of the subject should not affect GI values (Ha et al. 1992, Wolever 2004). Using the IAUC in the calculation also diminishes variation, and no significant correlation exists between GI and IAUC (Wolever 2004).

#### 2.4.2 Reference and test food

Glucose solution and white bread are generally used as reference foods in GI testing. Some studies also use rice or some other local starch-containing food as the reference (Sugiyama et al. 2003). In the study of Jenkins et al. (1981) the original reference food was a glucose solution, but shortly after launching the GI concept, white bread became more commonly used as a reference (Jenkins et al. 1983). The use of white bread has been advocated because it is closer to a physiological meal than a glucose solution (Wolever et al. 1985, Wolever et al. 1991). Moreover, subjects have complained that the sweetness of the glucose solution is nauseating. On the other hand, the high osmotic load caused by a glucose solution may lead to delayed gastric emptying, which may modify the results. White bread and other starchy foods contain fat and protein, while a glucose solution does not. As known, protein stimulates insulin secretion (Nuttall et al. 1984), which leads to greater insulin responses to starchy foods than to glucose solution despite lower glucose responses. Thus, one of the advantages of white bread is that the GI and II values are related (Bornet et al. 1987, Wolever et al. 1988b).

However, differences in the composition and digestibility characteristics of white bread from one experiment to another have been proposed to reduce its usability as a reference food (Bornet et al. 1987, Wolever et al. 2003). There have been contradictory findings regarding the variation of glucose responses when IAUCs of white bread and glucose solution have been compared. White bread and glucose solution have produced similar variation (Williams et al. 2008, Wolever et al. 2003) in contrast to the earlier findings where glucose solution produced 1.7 times higher variation than white bread (Wolever et al. 1996) (Table 1). In the interlaboratory study comparing the results of seven laboratories, the variability of GI values of white

bread was locally obtained and the portion size was based on local food tables, was similar to that of other foods provided (Wolever et al. 2003). However, a glucose solution should be used as the reference to ensure international standardization (Bornet et al. 1987, Wolever et al. 2003). Comparing different studies, the GIs are typically higher when white bread is used as the reference food. If white bread is used as the reference, the value should be multiplied by 0.71 for conversion to a glucose solution (FAO/WHO 1998).

**Table 1.** Coefficients of variation (CV) within subjects after intake of white bread and glucose solution<sup>1</sup>.

Reference		Replicates	White bread	Glucose solution	P
Wolever et al.	1996	3	12.7	21.4 <sup>2</sup>	ns
Wolever et al.	2003	3	27.7	23.1	ns
Williams et al.	2008	3 <sup>3</sup>	25.9	25.3	ns

<sup>1</sup> Amount of available carbohydrate 50 g

<sup>2</sup> Amount of available carbohydrate 75 g

<sup>3</sup> Replicates of glucose solution 4

The number of reference tests affects the variability of GI values (Table 2). To reduce variability, each subject should have at least three reference tests (Wolever et al. 1991). When only one test of the reference food was used to calculate the GI of the test food (Vega-Lopez et al. 2007, Wolever et al. 2003), it resulted in higher GI values and SDs than when GI was calculated using the mean of three tests, but contradictory findings have also been presented (Wolever et al. 1985). In a study where the reference food was tested several times in diabetic subjects, two additional tests did not improve accuracy. The GI values, SDs, and CVs did not improve when four or five reference tests were used ( $75.9 \pm 19.4$ , CV 26% and  $76.6 \pm 19.7$ , CV 26%, respectively) relative to the GI estimate with three reference tests ( $75.4 \pm 18.9$ , CV 25%) (Wolever et al. 1985). The FAO/WHO protocol (FAO/WHO 1998) advocates testing the reference food three times, but adherence to the protocol has not been systematic. As repeating the test of the reference food improves reliability, it is important to evaluate the required number of reference tests to find a balance between the accuracy of GI values, the costs of testing, and the burden to participants (Brouns et al. 2005).

The GI values of test foods are calculated as a ratio of the test food IAUC and the mean of reference food IAUCs. As a consequence, the reference food is the denominator of each test food in the experiment, but repeating measurements of the test food will also improve precision of GI estimates (Brouns et al. 2005). In a study where the test food was repeated once, the between-subject variation diminished from 19% to 17%, but there was no significant effect on GI value (GIs for rice

80.5±15.2, tested once, and 83.4±14.9, tested twice) (Wolever et al. 1985). Similar findings were demonstrated in a study where four different test meals (rice, mashed potato, pumpernickel, and rye bread) were tested twice (Wolever et al. 1989). The variation decreased, but there was no significant difference between GI values. In another study, Wolever et al. (1990) evaluated two test meals, rice and spaghetti, and the reference food, white bread, four times each. They found that when individual tests were compared, differences in GI obtained were mainly due to day-to-day variability within the same subject (Wolever et al. 1990). Thus, repeating the test meal will also diminish variation in measured GI values. Currently, however, there is no recommendation for repeating measurements of the test food.

**Table 2.** Glycaemic index (GI) and repeating the reference food.

Study	Subjects	n	Reference	Replicates	Mean±SD	CV%
Wolever et al. 1985	Type 2 DM	10	white bread	1	68±22 <sup>1</sup>	ng <sup>2</sup>
			white bread	2	72±20 <sup>1</sup>	ng
			white bread	3	74±20 <sup>1</sup>	ng
			white bread	4	74±20 <sup>1</sup>	ng
			white bread	5	74±19 <sup>1</sup>	ng
	Type 1 DM	6	white bread	1	76±28 <sup>1</sup>	ng
			white bread	2	80±25 <sup>1</sup>	ng
			white bread	3	77±19 <sup>1</sup>	ng
			white bread	4	79±18 <sup>1</sup>	ng
	Diabetic	16	white bread	5	82±22 <sup>1</sup>	ng
			white bread	1	71±24 <sup>1</sup>	ng
			white bread	2	75±22 <sup>1</sup>	ng
			white bread	3	75±19 <sup>1</sup>	25
			white bread	4	76±19 <sup>1</sup>	ng

Vega-López et al. 2007	Healthy	14	white bread	5	77±20 <sup>1</sup>	ng
			glucose solution	1	78±37 <sup>3</sup>	50
			glucose solution	2	69±19 <sup>3</sup>	25
			glucose solution	3	71±22 <sup>3</sup>	30
<hr/>						
<sup>1</sup> GI of rice						
<sup>2</sup> Not given						
<sup>3</sup> GI of white bread						

### 2.4.3 Coffee as a beverage in GI measurement

Caffeine ingested with a glucose solution has elicited greater blood glucose and insulin responses than glucose solution alone during an oral glucose tolerance test (OGTT) (Battram et al. 2006, Graham et al. 2001, Lane et al. 2004, Petrie et al. 2004, Thong and Graham 2002) (Table 3). Studies focusing on how caffeine in coffee affects postprandial glucose and insulin responses have produced inconsistent findings regarding postprandial glucose responses (Battram et al. 2006, Johnston et al. 2003, Pizziol et al. 1998) (Table 3).

Coffee is generally allowed as a drink in GI measurement, but the practice is not widely encouraged. Concerns have been expressed about the confounding effects of caffeine-containing beverages on GI measurement. Therefore, the recommendation is that subjects drink only water during GI measurement (Brouns et al. 2005). However, the effect of coffee on GI values remains open. In a previous study, coffee (250 ml) had no significant effect on glucose IAUCs of solid carbohydrate-containing test meals, but significantly increased the blood glucose concentration at 30 or 45 min compared with water (Aldughpassi and Wolever 2009b, Young and Wolever 1998).

**Table 3.** Effect of caffeine and coffee on glucose and insulin responses.

Study		Glycaemia	P	Insulinaemia	P
Graham et al.. 2001	Caffeine	24% ↑	ns	60% ↑	≤0.001
Thong&Graham 2002	Caffeine	-	ns	38% ↑	<0.05
Lane et al.. 2004	Caffeine	21% ↑	0.04	48% ↑	0.01
Petrie et al.. 2004	Caffeine	10% ↑	ns	14% ↑	≤0.05
Battram et al.. 2006	Caffeine	55% ↑	≤0.05	48% ↑	≤0.05
Pizziol et al.. 1998	Coffee	10% ↑	<0.001	2% ↑	ns
Johnston et al.. 2003	Coffee	1% ↑	ns	7% ↑	ns
Battram et al.. 2006	Coffee	4% ↓	ns	21% ↑	ns

↑ =increase compared with caffeine capsules or decaffeinated coffee

↓ =decrease compared with caffeine capsules or decaffeinated coffee

## 2.5 Effects of subjects' characteristics on GI values

Subjects' characteristics, including age, sex, body mass index (BMI), and ethnicity, influence postprandial glycaemia. Differences in postprandial glycaemia are also due to differences in insulin sensitivity and secretion (Pi-Sunyer 2002). People who require a normal amount of insulin to process glucose are insulin-sensitive. Insulin resistance indicates an abnormal physiological response to insulin in its target tissues. In insulin-resistant subjects, postprandial glucose uptake is promoted by two factors, hyperinsulinaemia and hyperglycaemia. Hyperinsulinaemia will compensate impaired insulin action, and hyperglycaemia promotes tissue glucose uptake. Postprandial insulin response can be almost twofold higher in insulin-resistant subjects relative to insulin-sensitive subjects (Galgani and Ravussin 2012).

One of the concerns regarding GI has been that GI values are inconsistent due to variation between individuals, but no recommendation has been given regarding subjects' background (FAO/WHO 1998). Reports have indicated that GI values are not significantly related to gender or ethnicity (Henry et al. 2008, Wolever et al. 2003, Wolever et al. 2008), but contradictory findings have also been described (Kataoka et al. 2013, Venn et al. 2010, Wolever et al. 2009). While the prevalence of impaired fasting glucose and diabetes is known to increase with age (Cowie et al. 2010), it has been suggested that GIs are not affected by age (Wolever et al. 1988a, Wolever et al. 2008, Wolever et al. 2009). The GI values of lentils measured among diabetic children and adults were virtually identical (Wolever et al. 1988a). In a recent study, however, older age increased postprandial glycaemia, resulting in a 25% higher GI value of cornflakes ( $P=0.008$ ) compared with in a group of younger subjects (Venn et al. 2011).

Knowledge is still lacking regarding the mean and variation of GI data measured are in insulin-resistant or overweight subjects relative to the data measured in healthy subjects (Brouns et al. 2005).

### 2.5.1 Glucose tolerance and insulin sensitivity

Subjects' glucose tolerance influences their glucose and insulin responses. Postprandial glucose responses are greater in diabetic subjects and subjects with impaired glucose tolerance (IGT) than in subjects with normal glucose tolerance. Disease duration also affects insulin secretion; in long-standing type 2 diabetes insulin, secretion is usually reduced (Taylor 2013). Use of subjects with normal glucose tolerance has been recommended in GI testing because the variability in glycaemic response is greater in subjects with impaired glucose tolerance (Brouns et al. 2005). However, expressing glucose response to a tested food as a percentage of glucose response to the reference food should lead to similar GI values despite the subjects' characteristics. In other words, the factors that affect glucose and insulin responses may not necessarily affect GI. However, the studies that have determined



whether GI values are affected by subjects' characteristics have compared healthy subjects with type 2 diabetic subjects (only two studies) or compared type 2 diabetic subjects with type 1 diabetic subjects (Jenkins et al. 1984, Jenkins et al. 1986, Wolever et al. 1985, Wolever et al. 1986, Wolever et al. 1987, Wolever et al. 1989) (Table 4). The GI values determined in subjects with diabetes or impaired glucose tolerance were separated from the values in healthy subjects in the latest international GI database (Atkinson et al. 2008). However, there are only a few studies that have assessed the effects of glucose tolerance and insulin sensitivity on GI values (Lan-Pidhainy and Wolever 2011, Wolever et al. 1998a) (Table 4). In addition, in many of the above-mentioned studies, the number of subjects was less than 10, which is the minimum number of subjects required for qualified GI studies (ISO 26642:2010. 2010). Further research is needed to clarify the effects of glucose tolerance on measured GI values.

**Table 4.** Effect of glucose tolerance status on glycaemic index (GI) values.

Study	n	Status	Reference food	Study food	GI		P
					mean±SD	CV%	
Jenkins et al. 1984	6	T1DM	White bread	White pea beans	84±24	29	not given
	7	T2DM	White bread	White pea beans	41±13	32	
Wolever et al. 1985	6	T1DM	White bread	Rice	82±22	27	ns
	10	T2DM	White bread	Rice	74±19	26	
Jenkins et al. 1986	4	T1DM	White bread	Whole-grain wheat bread	98±10	10	ns
	4	T2DM	White bread	Whole-grain wheat bread	91±28	31	
	5	T1DM	White bread	Whole-grain rye bread	87±22	25	ns
	4	T2DM	White bread	Whole-grain rye bread	100±34	34	
	5	T1DM	White bread	Pumpemickel (rye) bread	76±16	21	ns
	4	T2DM	White bread	Pumpemickel (rye) bread	86±10	12	
	5	T1DM	White bread	Bulgur	65±22	34	ns
	6	T2DM	White bread	Bulgur	68±20	29	
	6	T1DM	White bread	Wheat kernels	67±20	30	ns
	6	T2DM	White bread	Wheat kernels	69±20	29	
Wolever et al. 1986	5	T1DM	White bread	Rye kernels	58±18	31	ns
	4	T2DM	White bread	Rye kernels	51±24	47	
	5	T1DM	White bread	Regular rice	77±9	19	ns
	13	T2DM	White bread	Regular rice	86±6	21	
	5	T1DM	White bread	Parboiled rice	64±7	16	ns
	13	T2DM	White bread	Parboiled rice	68±6	21	
Wolever et al. 1987	5	T1DM	White bread	Whole-grain wheat bread	98±12	12	

Wolever et al. 1989	11	T2DM	White bread	Whole-grain wheat bread	95±23	24	ns
	6	T1DM	White bread	Whole-grain rye bread	94±25	27	
	9	T2DM	White bread	Whole-grain rye bread	90±22	24	ns
	6	T1DM	White bread	Pumpemickel bread	88±32	36	
	9	T2DM	White bread	Pumpemickel bread	79±10	13	ns
Wolever et al. 1989	6	T1DM	White bread	Pumpemickel	87±27	31	
	5	T2DM	White bread	Pumpemickel	80±12	15	ns
	6	T1DM	White bread	Rye bread	86±18	21	
	5	T2DM	White bread	Rye bread	86±14	16	ns
Wolever et al. 1998	10	Healthy	Glucose	Diabetes screening product	51±19	37	
	9	IGT	Glucose	Diabetes screening product	52±12	23	
	8	T2DM	Glucose	Diabetes screening product	54±6	11	ns
Lan-Pidhainy et al. 2011	9	Healthy	Glucose	Sucrose	68±27	40	
	12	Hyperinsulinaemic <sup>1</sup>	Glucose	Sucrose	69±21	30	
	10	T2DM	Glucose	Sucrose	68±16	24	0.95
	9	Healthy	Glucose	Mashed potato	101±21	21	
	12	Hyperinsulinaemic <sup>1</sup>	Glucose	Mashed potato	81±35	43	
	10	T2DM	Glucose	Mashed potato	98±16	16	0.20
	9	Healthy	Glucose	White bread	63±33	52	
	12	Hyperinsulinaemic <sup>1</sup>	Glucose	White bread	70±21	30	
	10	T2DM	Glucose	White bread	71±19	27	0.13
	9	Healthy	Glucose	Rice	61±21	34	
	12	Hyperinsulinaemic <sup>1</sup>	Glucose	Rice	72±24	33	
	10	T2DM	Glucose	Rice	70±32	46	0.10
	9	Healthy	Glucose	Barley	58±18	31	
	12	Hyperinsulinaemic <sup>1</sup>	Glucose	Barley	44±21	48	
	10	T2DM	Glucose	Barley	47±16	34	0.55

<sup>1</sup>Hyperinsulinaemic= fasting serum insulin ≥40 pmol/l (This cut-off point was chosen because it represents approximately the 67<sup>th</sup> percentile for non-diabetic subjects in the laboratory of Wolever et al. (Lan-Pidhainy and Wolever 2011)).

### 2.5.2 Body weight

A strong association exists between overall obesity and risk of type 2 diabetes (Chan et al. 1994). Obesity increases insulin response by enhancing insulin secretion due to insulin resistance (Elahi et al. 1982), and also decreases insulin clearance (Jones et al. 2000, Meistas et al. 1983). Obese individuals tend to have higher insulin responses to a 75-g oral glucose challenge (Kim and Reaven 2008), but there are only a few studies have focused on the effect of body weight on GI values (Table 5). Overweight and obese subjects tended to produce smaller GI values than their leaner peers (Wolever et al. 2009, Wolever et al. 1998b), but the variation was smaller in lean subjects (Table 5). Based on the results of two interlaboratory studies, the subject's BMI appears not to significantly alter GI values (Wolever et al. 2003, Wolever et al. 2008), but more studies are needed to confirm this (Brouns et al. 2005).

**Table 5.** Effect of body weight on glycaemic index (GI) values.

Study	<i>n</i>		Status	Reference food	Study food	GI		<i>P</i>
						mean±SD	CV%	
Wolever et al. 1998	10	Lean	Glucose		Diabetes screening product	51±19	37	
	9	Obese	Glucose		Diabetes screening product	41±12	29	ns
Wolever et al. 2009	37	Lean	Glucose		White bread	68±18	26	
	40	Overweight	Glucose		White bread	76±25	33	ns
	37	Lean	Glucose		Chocolate-chip cookie	43±18	42	
	40	Overweight	Glucose		Chocolate-chip cookie	41±19	46	<0.05
	37	Lean	Glucose		Fruit leather	36±12	33	
	40	Overweight	Glucose		Fruit leather	30±13	43	ns

## 2.6 Macronutrients and glycaemic index

As foods are seldom eaten alone, it is essential for the GI to also apply well to mixed meals. However, glycaemic and insulinaemic responses to mixed meals are modified by the amount of macronutrients in the meals. According to Wolever et al. (2006a), carbohydrate content and GI together explain about 90% of the variation of the glycaemic responses to mixed meals, and fat and protein have only negligible effects on glycaemic response. On the other hand, a study by Flint et al. (2004) suggested that the GI value of a meal is more strongly correlated with either the fat or protein content than the carbohydrate content alone. Regarding insulin responses, Wolever et al. (2006a) observed a strong correlation between the glycaemic and insulinaemic responses to mixed meals. However, mixed meals with similar carbohydrate contents induce a wide range of insulin responses, and the fat content of a mixed meal has a significant inverse relation with the insulinaemic responses (Bao et al. 2009).

### 2.6.1 Fat

Fat reduces glycaemic responses by delaying gastric emptying and enhancing the secretion of incretins such as GLP-1 and GIP (Collier and O'Dea 1983, Deane et al. 2010, Gannon et al. 1993a, Gannon et al. 1993b, Gentilcore et al. 2006, Owen and Wolever 2003, Simpson et al. 1985a, Welch et al. 1987). Fat also reduces insulin responses (Gannon et al. 1993a, Gulliford et al. 1989, Welch et al. 1987).

When fat is ingested with carbohydrate-containing foods, glycaemic responses flatten and diminish, and hence, the overall GI is lower, but differences in GI values were not significant (Henry et al. 2006). Contrary findings also exist (Leeman et al. 2008) (Table 6). Dose-response effects of fat on glycaemic responses have been noted (Moghaddam et al. 2006), but different degrees of saturation of added fat did not affect glycaemic responses similarly (MacIntosh et al. 2003). The ability of fat to decrease glycaemic responses may be diminished in insulin-resistant subjects (Moghaddam et al. 2006) and in type 2 diabetic subjects (Gannon et al. 1993a).

### 2.6.2 Protein

Protein ingested with carbohydrates increases insulin responses, which leads to reduced glycaemia (Krezowski et al. 1986, Nuttall et al. 1984, Spiller et al. 1987). In addition, ingested protein slows gastric emptying by increasing the secretion of GIP, cholecystokinin (CCK), peptide YY (PYY), and GLP-1 (Jahan-Mihan et al. 2011, Karamanlis et al. 2007). Slower gastric emptying causes reduced glycaemic

responses. Protein also suppresses ghrelin secretion (El Khoury et al. 2010). Ghrelin, in contrast to other gut peptides, stimulates gut motility. In addition, protein stimulates glucagon secretion which promotes glycogenolysis and gluconeogenesis, counteracting any insulin-induced decline in glucose levels (Schmid et al. 1992). Moreover, different proteins have different effects on insulin responses, which is likely due to a potentiating effect of amino acids on the  $\beta$ -cell. Proteins that are rich in the branched-chain amino acids leucine, valine, and isoleucine are particularly associated with enhanced insulin response (Gannon et al. 1988, Nilsson et al. 2004, van Loon et al. 2000).

Protein influences glucose and insulin responses in a dose-dependent manner (Gunnerud et al. 2013). The addition of protein into a carbohydrate-rich meal has been found to decrease GI values (Table 6), but at least 30 g of protein is needed to cause a significant effect (Moghaddam et al. 2006). Only a few studies have examined the effect of protein on GIs of solid foods (Bornet et al. 1987, Henry et al. 2006).

**Table 6.** Effect of fat and protein on glycaemic index (GI) values.

Study	n	Status	Reference food	Study food	GI		P
					mean±SD	CV%	
Bornet et al. 1987	18	T2DM	Glucose	White bread	95±15		ns
	18	T2DM	Glucose	White bread + cheese (protein 30 g) + butter (fat 20 g)	~20% ↓		
	18	T2DM	Glucose	Potato	74±12		ns
	18	T2DM	Glucose	Potato + cheese (protein 30 g) + butter (fat 20 g)	~20% ↓		
	18	T2DM	Glucose	Spaghetti	64±15		ns
	18	T2DM	Glucose	Spaghetti + cheese (protein 30 g) + butter (fat 20 g)	~20% ↓		
	18	T2DM	Glucose	Rice	56±2		ns
	18	T2DM	Glucose	Rice + cheese (protein 30 g) + butter (fat 20 g)	~20% ↓		
	18	T2DM	Glucose	Lentils	30±15		ns
	18	T2DM	Glucose	Lentils + cheese (protein 30 g) + butter (fat 20 g)	~20% ↓		
MacIntosh et al. 2003	18	T2DM	Glucose	Beans	23±1		ns
	18	T2DM	Glucose	Beans + cheese (protein 30 g) + butter (fat 20 g)	~20% ↓		
	10	Healthy	Glucose	Instant mashed potato + sunflower oil (30 g; PUFA <sup>1</sup> 64%)	68±25	37	ns
	10	Healthy	Glucose	Instant mashed potato + butter (30 g; SFA <sup>2</sup> 69%)	74±32	43	
Henry et al. 2006	10	Healthy	Glucose	Potato	93±25	27	



Moghaddam et al. 2006	10	Healthy	Glucose	Potato + cheddar cheese (120 g)	39±16	41	ns
	10	Healthy	Glucose	Potato + tuna (120 g)	76±22	29	
	10	Healthy	Glucose	Pasta	61±28	46	
	10	Healthy	Glucose	Pasta + cheddar cheese (120 g)	27±13	48	ns
	10	Healthy	Glucose	Pasta + tuna (120 g)	28±9	32	
	10	Healthy	Glucose	White toast	50±22	44	
	10	Healthy	Glucose	White toast + cheddar cheese (120 g)	35±6	17	ns
	20	Healthy	Glucose	Glucose	100±9	9	<0.05
	20	Healthy	Glucose	Glucose + corn oil (5 g)	106±27	27	
	20	Healthy	Glucose	Glucose + corn oil (10 g)	100±27	27	
	20	Healthy	Glucose	Glucose + corn oil (30 g)	99±22	22	
	20	Healthy	Glucose	Glucose + soy protein (5 g)	96±22	23	
	20	Healthy	Glucose	Glucose + corn oil (5 g) + soy protein (5 g)	106±27	25	
	20	Healthy	Glucose	Glucose + corn oil (5 g) + soy protein (5 g)	91±31	34	
	20	Healthy	Glucose	Glucose + corn oil (10 g) + soy protein (5 g)	88±31	35	
	20	Healthy	Glucose	Glucose + corn oil (30 g) + soy protein (5 g)	94±22	23	
	20	Healthy	Glucose	Glucose + corn oil (0 g) + soy protein (10 g)	98±36	37	
	20	Healthy	Glucose	Glucose + corn oil (5 g) + soy protein (10 g)	87±22	25	
	20	Healthy	Glucose	Glucose + corn oil (30 g) + soy protein (10 g)	72±27	36	

20	Healthy	Glucose	Glucose + corn oil (0 g) + soy protein (30 g)	68±18	26	<0.05
20	Healthy	Glucose	Glucose + corn oil (5 g) + soy protein (30 g)	63±13	21	<0.05
20	Healthy	Glucose	Glucose + corn oil (10 g) + soy protein (30 g)	61±22	36	<0.05
20	Healthy	Glucose	Glucose + corn oil (30 g) + soy protein (30 g)	57±31	54	<0.05
Leeman et al. 2008	14 Healthy	White bread	Potato	111±51	46	
	14	White bread	Potato + sunflower oil (15.4 g)	131±55	42	ns

<sup>1</sup>PUFA = polysaturated fatty acids

<sup>2</sup>SFA = saturated fatty acids

### 2.6.3 Alcohol

Epidemiological studies have shown an association between moderate alcohol consumption and improved glucose tolerance and insulin sensitivity (Carlsson et al. 2000, Facchini et al. 1994, Kiechl et al. 1996, Mayer et al. 1993). The impact of alcohol on glucose homeostasis has also been investigated in short-term intervention studies lasting from 2 weeks to 3 months, but their results are inconsistent (Davies et al. 2002, Kim et al. 2009, Napoli et al. 2005, Shai et al. 2007, Zilkens et al. 2003). Alcohol acutely inhibits gluconeogenesis in the liver (Yki-Järvinen and Nikkilä 1985). Acute alcohol consumption also causes insulin resistance, whereas chronic alcohol consumption may improve insulin sensitivity (Ting and Latt 2006).

Only a few studies have focused on postprandial responses to beer (Table 7). When non-alcoholic beer was compared with regular beer, postprandial glucose responses tended to decrease, but insulin responses increased (Christiansen et al. 1993). However, when beer was ingested with white bread, the results were controversial (Brand-Miller et al. 2007, Christiansen et al. 1994).

The carbohydrate contents of alcoholic beverages, including beer, are so low that it is difficult to measure their GI values using standard methods (Brand-Miller et al. 2007). Thus, no study testing the GI of beer by standard methodology has been published to date in a peer-reviewed journal. As a consequence, epidemiological studies focusing on the association between GI and chronic diseases have used highly variable GI values for beer, ranging from 36 to 95 (Flood et al. 2006, Neuhauser et al. 2006, Schulz et al. 2005). These imputed values have been deduced from carbohydrate-rich beverages such as milk and orange juice.

**Table 7.** Postprandial glucose and insulin responses of beer.

Study		Status	Food	Time	Glycaemia mmol/l mean±SD	Insulinaemia pmol/l mean±SD	P
	<i>n</i>						
Christiansen et al. 1993	10	T2DM	Nonalcoholic beer (500 ml)	4h	395±187	5430±3662	ns ns <0.01
			Beer (500 ml, v/v of 2.7)	4h	365±272	9336±6868	
			Beer (500ml, v/v of 5.4)	4h	261±82	12336±9240	
Christiansen et al. 1994	10	T2DM	Nonalcoholic beer (500 ml) + white bread (100g) + ham (40 g) + low-fat margarine (20 g)	4h	554±218	6716±2795	ns
			Nonalcoholic beer (500 ml) + alcohol (24 g) + white bread (100g) + ham (40 g) + low-fat margarine (20 g)	4h	574±281	8252±3773	
Brand-Miller et al. 2007	10	Healthy	White bread as the reference (97 g) = 1000kJ	2h	~150	~12000	<0.001
			Beer (667 g) = 1000 kJ	2h	~75	~3000	
Brand-Miller et al. 2007	10	Healthy	White bread as the reference (198 g) + margarine (10 g)	3h	~325	~22500	ns
			Beer (667 g) + white bread (198 g) + margarine (10 g)	3h	~250	~22500	

### 3 Aims of the study

The objective of this thesis was to examine the effect of methodological choices on glycaemic index (GI) and how glucose tolerance, other macronutrients, and beverages modify the index. Glycaemic responses were determined in order to assess the index. Specific aims were to answer the following questions:

- How do choice of blood sampling method, choice of the reference food, and number of reference food tests conducted affect GI values (Study I)?
- Does coffee co-ingested with carbohydrates affect GI values (Study II)?
- Do glucose tolerance and body weight of subjects tested affect GI values (Study III)?
- What is the impact of co-ingested fat and protein on GI values (Study IV)?
- How does alcohol modify GI values (Study V)?



# 4 Subjects and methods

## 4.1 Study designs

All studies were conducted at the National Institute for Health and Welfare (former National Public Health Institute) in 2004 (*Studies I and IV*), 2005 (*Study II*), 2007 (*Study III*), and 2008 (*Study V*). The postprandial tests were performed in the morning following an overnight fast. The tests were conducted in random order with approximately one week between each test.

The subjects were requested to follow their usual diet during the study. They were advised to consume at least 150 g of carbohydrates daily during the three days before the study day. Baseline data on diet, health, and lifestyle were assessed by questionnaires. Subjects' mean energy intake was calculated on the basis of their estimated basal metabolic rate, taking into account their physical activity. This information was used to compose individual standardized meals for the evening preceding the study day. The energy content of the evening meal amounted to 15% of the daily energy needs of each individual, and the proportion of the energy from carbohydrates was 55%. The subjects were advised to avoid vigorous physical activity and not to consume alcohol on the day preceding the study day.

All postprandial studies were performed in the morning after a 10- to 12-h overnight fast. To avoid exercise on the study mornings, the subjects were advised to arrive by car or by public transportation. Every study morning, body weight was measured. Changes of up to 2 kg in weight were allowed during the study, and no weight changes of over 2 kg were observed.

Qualified nurses performed blood sampling. In *Study I*, upon arrival at the study site, an intravenous catheter was inserted into an antecubital vein in the subject's forearm, and a baseline venous blood sample was drawn. Thereafter, a baseline finger-prick capillary blood sample was taken. Next, the subjects consumed a study meal within 10 min. In *Study I*, venous and finger-prick capillary blood samples were obtained at 15, 30, 45, 60, 90, 120, and 180 min after the start of the meal. Because the capillary sample was obtained first, the actual sampling time of the intravenous sample was recorded. In *Studies II-V*, a baseline capillary blood sample was taken from a fingertip. Fasting capillary glucose was measured in duplicate from the same fingertip and the mean of these measurements was applied in *Studies III and V*. Thereafter, the subject consumed the study meal within 10 min, and capillary blood samples were collected at 15, 30, 45, 60, 90, and 120 min. The subjects were asked to avoid physical activity for the duration of the tests.

All postprandial studies were conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were

approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa. Written informed consent was obtained from all subjects prior to start of the studies.

## 4.2 Subjects

Healthy women and men for *Studies I, II, IV, and V* were recruited from staff of the National Institute for Health and Welfare (former National Public Health Institute) and the students of the University of Helsinki by internal mail and announcements in the personnel restaurant. The subjects of *Study III* were recruited from a subgroup of the population of the Helsinki Birth Cohort Study (HBCS) (Forsén et al. 1997). The HBCS study population (born during 1934-1944) was used to ensure finding enough subjects with impaired glucose tolerance.

In *Studies I, II, IV, and V*, the primary inclusion criterion was normal glucose tolerance following a 75-g oral glucose tolerance test (OGTT; fasting glucose <7 mmol/l and 2-h glucose concentration <7.8 mmol/l) (WHO 1999). Exclusion criteria in all studies included smoking, a first-degree family history of diabetes mellitus, and regular medication (oral contraceptives were allowed). For women, other exclusion criteria were pregnancy, breast feeding, a history of gestational diabetes, or polycystic ovary syndrome. In addition, in *Study V*, blood donation less than 90 days before the study was an exclusion criterion.

Background characteristics of the subjects in *Studies I, II, IV, and V* are presented in Table 8. At the screening visit, weight and height were measured with subjects wearing light indoor clothing and without shoes. Body mass index (BMI) was calculated as weight (kg) divided by height (m) × height (m). There was female predominance in all studies, and the participants of *Study V* were older and heavier. Mean fasting glucose and insulin levels were similar in all studies, as was insulin resistance calculated by a homeostasis model assessment of insulin resistance (HOMA1-IR) (Matthews et al. 1985).

**Table 8.** Characteristics of the subjects in *Studies I, II, IV, and V* (mean±SD).

	Study I	Study II	Study IV	Study V
Subject, n (M/F) <sup>1</sup>	12 (1/11)	12 (1/11)	12 (3/9)	10 (1/9)
Age, years	30.8±7.8	34.8±10.4	36.2±14.1	40.9±11.5
BMI <sup>2</sup> , kg/m <sup>2</sup>	21.4±1.7	21.9±2.5	21.3±1.7	23.0±3.3
fP <sup>3</sup> -Glucose, mmol/l	5.2±0.5	4.7±0.4	4.8±0.4	5.3±0.3
fS <sup>4</sup> -Insulin, mU/l	2.6±1.5	4.0±1.8	4.3±2.7	5.4±1.9
HOMA1-IR <sup>5</sup>	0.57±0.3	0.82±0.4	0.93±0.6	1.3±0.5

<sup>1</sup>M=males, F=females

<sup>2</sup>BMI=body mass index

<sup>3</sup>fP=fasting plasma

<sup>4</sup>fS=fasting serum

<sup>5</sup>Fasting plasma insulin (mU/l) × Fasting plasma glucose (mmol/l) / 22.5 (Matthews et al. 1985)



*Study III* included 24 normal-weight and 24 overweight subjects, with BMI of  $<25 \text{ kg/m}^2$  and  $27.5\text{--}34.9 \text{ kg/m}^2$ , respectively. The subjects were also screened by an OGTT, and based on this each weight group included 12 subjects with normal glucose tolerance (NGT) and 12 subjects with impaired glucose tolerance (IGT, fasting glucose  $<7.8 \text{ mmol/l}$  and 2-h glucose  $7.8\text{--}11.0 \text{ mmol/l}$ ). Exclusion criteria included a first-degree family history of diabetes mellitus, regular medication that would have an effect on glucose or lipid metabolism, gastrointestinal disease influencing absorption, milk allergy, and smoking.

Background characteristics of the subjects in *Study III* are shown in Table 9. Mean age and fasting glucose level were similar in the study subgroups. Fasting insulin was significantly higher in the overweight subjects than in normal-weight subjects, but IGT only non-significantly increased the fasting insulin level. HOMA1-IR followed the variation in insulin level.

**Table 9.** Characteristics of the subjects in *Study III* (mean $\pm$ SD).

	Normal-weight subjects (BMI $<25 \text{ kg/m}^2$ )		Overweight subjects (BMI $27.5\text{--}34.9 \text{ kg/m}^2$ )	
	NGT <sup>1</sup>	IGT <sup>2</sup>	NGT <sup>1</sup>	IGT <sup>2</sup>
Subject, n (M/F) <sup>3</sup>	12 (6/6)	12 (5/7)	12 (6/6)	12 (4/8)
Age, years	65.9 $\pm$ 2.5	66.7 $\pm$ 2.0	65.8 $\pm$ 2.4	66.3 $\pm$ 3.3
BMI <sup>4</sup> , $\text{kg/m}^2$	23.8 $\pm$ 1.5	23.1 $\pm$ 1.8	30.1 $\pm$ 1.8	30.8 $\pm$ 2.2
fP <sup>5</sup> -Glucose, mmol/l	5.4 $\pm$ 0.4	5.4 $\pm$ 0.5	5.5 $\pm$ 0.6	5.6 $\pm$ 0.4
2-h glucose, mmol/l	6.4 $\pm$ 0.9	9.0 $\pm$ 0.9	6.7 $\pm$ 1.0	9.2 $\pm$ 1.0
fS <sup>6</sup> -Insulin, mU/l	4.8 $\pm$ 1.4	5.9 $\pm$ 3.0	7.9 $\pm$ 5.7	8.9 $\pm$ 3.4
HOMA1-IR <sup>7</sup>	1.15 $\pm$ 0.4	1.38 $\pm$ 0.7	1.98 $\pm$ 1.6	2.26 $\pm$ 0.9

<sup>1</sup>NGT = normal glucose tolerance: fasting glucose  $<7.0 \text{ mmol/l}$  and 2-h glucose concentration after a 75-g glucose load  $<7.8 \text{ mmol/l}$

<sup>2</sup>IGT = impaired glucose tolerance: fasting glucose  $<7.8 \text{ mmol/l}$  and 2-h glucose  $7.8\text{--}11.0 \text{ mmol/l}$

<sup>3</sup>M=males, F=females

<sup>4</sup>BMI=body mass index

<sup>5</sup>fP=fasting plasma

<sup>6</sup>fS=fasting serum

<sup>7</sup>Fasting plasma insulin (mU/l)  $\times$  Fasting plasma glucose (mmol/l) / 22.5 (Matthews et al. 1985)

### 4.3 Study foods, meals, and nutrient compositions

*Studies I, II, and V* tested individual food items, and *Studies III and V* tested mixed meals. In each study, the foods and mixed meals were served to each subject in a random order one week apart.

In *Studies I-IV*, the study meals were given as a portion providing 50 g of available carbohydrates. In *Study V*, the study meals were given as a portion providing 25 g of available carbohydrate to avoid an unrealistically large beverage volume for consumption within 10 min. The amounts of available carbohydrates were based on chemical analysis. In *Studies I-IV*, the reference glucose solution was prepared by dissolving 50 g of D-glucose powder (Yliopiston Apteekki, Finland) in 250 ml of tap water. In *Study V*, the amount of glucose powder was 25 g, and it was also dissolved in 250 ml of tap water. The total water volume of all meals was standardized to 500 ml in *Study I* and to 550 ml in *Studies II-V* by adjusting the water or volume of the beverage.

#### 4.3.1 Rye bread, instant mashed potato, and oatmeal porridge

In *Study I*, three test foods, whole-grain rye bread (whole-grain rye flour 69%, Jälkiuunileipä, Oululainen Ltd., Finland), instant mashed potato (Idahoan Foods, Lewisville, ID, USA), and oatmeal porridge (Elovena, Raisio Group Ltd., Finland), were evaluated. White bread (wheat flour 100%, Ranskanleipä, Vaasan&Vaasan Ltd., Finland) and glucose solution (Yliopiston Apteekki, Finland) were used as the reference meals. Oatmeal porridge and instant mashed potato were prepared according to package directions, except that the mashed potato was prepared with water instead of milk.

Each test food was tested once, and both reference foods were tested three times. The study foods were served with 40 g of cucumber (except oatmeal porridge and glucose solution). The subjects had the choice of water or a non-caloric orange beverage for consumption with the study food throughout the study. Of the 12 subjects, nine chose water and three chose the non-caloric beverage.

The chemical composition of the study foods and the evening meals was analysed by the VTT (Technical Research Centre of Biotechnology, Espoo, Finland). The protein content was estimated ( $N \times 6.25$ ) from the quantitative analysis of nitrogen by the Kjeldahl method (Eagan 1981). The fats were determined gravimetrically by extraction in diethyl ether and petroleum ether after hydrolysis with acid. Total fibre and soluble and insoluble fibres were determined by the Asp method (Asp et al. 1983). Free sugars (i.e. glucose, fructose, maltose, maltotriose, and sucrose) were measured by using an ion chromatograph system (Dionex, Sunnyvale, CA, USA). Furthermore, the enzymatically available starch contents of the study meals and evening meals were analysed by the method proposed by McCleary et al. (1997) using the assay kit of Megazyme (Wicklow, Republic of

Ireland). The available carbohydrate was calculated as the sum of free sugars and enzymatically available starch. The nutrient composition of the study foods is shown in Table 10.

#### 4.3.2 Mashed potato-based meals

In *Study IV*, the subjects were given six different mashed potato-based meals (Van Gogh, prepared with water and margarine) once and the reference glucose solution twice. Each of the test meals and the reference food were given as portions, providing 50 g of available carbohydrates, except three of the meals that included salad as portions, providing about 54 g of available carbohydrates. Study meals were served with water and 40 g of cucumber, except for the meals including salad.

The nutrient composition of the mashed potato-based study meals was analysed by VTT (Technical Research Centre of Biotechnology, Espoo, Finland) similarly to *Study I*. The amount of available carbohydrates was calculated as the sum of the free sugars and the enzymatically available starch. The nutrient composition of the study meals is shown in Table 10.

#### 4.3.3 Coffee

In *Study II*, two different coffee portions (Juhlamokka, Gustav Paulig Ltd., Finland) were tested: a small coffee (125 ml) containing 150 mg of caffeine and a large coffee (250 ml) containing 300 mg of caffeine, both portions together with 250 ml of water containing 50 g of available carbohydrate. The twice-tested reference food was given as a portion providing 50 g of available carbohydrate.

The caffeine content of the coffees was analysed by the National Institute for Health and Welfare, using a method (Oikarinen et al. 2007) based on liquid-liquid extraction and high-performance liquid chromatography, HPLC (Agilent Eclipse XDP-C18, Palo Alto, CA, USA), and measured with a UV detector (Agilent 1100 Series, Palo Alto, CA, USA). The nutrient composition and caffeine content of the study beverages are shown in Table 10.

#### 4.3.4 Alcohol-containing beverages

In *Study V*, three test beverages, glucose solution with alcohol, non-alcoholic beer (Nikolai Lager, 0.0%vol, Sinebrychoff Ltd., Kerava, Finland), and beer (Nikolai Lager, 4.5%vol, Sinebrychoff Ltd., Kerava, Finland), were tested. Each of the test beverages and the twice-tested reference glucose solution were given as portions providing 25 g of available carbohydrates. The volume of non-alcoholic beer and beer was 640 ml and 510 ml, respectively, and that of glucose solution with and without alcohol was 500 ml. Both the glucose solution with alcohol and the beer

provided 21 g of ethanol per portion. The glucose solution with alcohol was prepared by mixing 25 g of glucose and 21 g of alcohol (Spiritus Fortis A, Ethanolum, 96%, Berner Ltd., Helsinki, Finland) in 250 ml of water and served with 250 ml of water.

The nutrient composition of the study beverages was analysed by AnalyCen laboratory (Lidköping, Sweden). Starch was analysed by using an amyloglucosidase/ $\alpha$ -amylase method (AOAC 996.11) (Association of Official Analytical Chemists 2003). Free sugars were measured by using an ion chromatograph system (Dionex, Sunnyvale, CA, USA). The malto-oligosaccharides of the study beverages were analysed by Eurofins Food (Eurofins Food B.V., Heerenveen, the Netherlands). The content of different malto-oligosaccharides was determined with high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using external standards. The nutrient composition of the study beverages is shown in Table 10.

**Table 10.** Nutrient composition of study meals in *Studies I, II, IV, and V*.

	Weight g	Energy kJ/portion	ACHO <sup>1</sup> g/portion	Starch g/portion	Sugars g/portion	Protein g/portion	Fat g/portion	Fibre g/portion
<b>Study I</b>								
White bread <sup>2</sup>	98	1078	50	45.6	4.4	8.5	2.2	3.4
Rye bread <sup>3</sup>	110	1109	50	47.2	2.8	10.3	2.2	13.6
Oatmeal porridge <sup>4</sup>	495	1321	50	49.0	1.0	13.4	6.4	10.9
Instant mashed potato <sup>5</sup>	370	1561	50	48.5	1.5	6.3	15.9	7.4
<b>Study II</b>								
Glucose solution with small coffee <sup>6</sup>	175	862	50	-	50	0.7	<0.25	-
Glucose solution with large coffee <sup>7</sup>	300	862	50	-	50	0.7	<0.25	-
<b>Study IV</b>								
Mashed potato <sup>8</sup>	362	1063	50.0	48.5	1.4	4.7	3.6	4.7
Mashed potato with oil <sup>9</sup>	392	2173	50.0	48.5	1.4	4.7	33.6	4.7
Mashed potato with chicken breast <sup>10</sup>	470	1825	50.1	48.5	1.5	34.6	10.4	4.7

Mashed potato with salad <sup>11</sup>	482	1150	53.7	49.6	4.0	5.9	3.7	6.0
Mashed potato with oil, chicken breast, and salad <sup>12</sup>	620	3022	53.8	49.6	4.1	35.8	40.5	6.0
Mashed potato with oil, chicken breast, salad, and rye bread <sup>13</sup>	560	3138	53.7	49.6	4.4	37.5	44.6	7.6

### Study V

Glucose solution with alcohol <sup>14</sup>	321	1034	25	-	25	-	-	-
Non-alcoholic beer <sup>15</sup> 0.0%vol	640	425	25	13.4	11.6 <sup>17</sup>	-	-	-
Beer <sup>16</sup> , 4.5%vol	510	1034	25	15.8	9.2 <sup>17</sup>	-	-	-

<sup>1</sup>ACHO, Available carbohydrates

<sup>2</sup>White bread (Ranskanleipä, Vaasan&Vaasan Ltd.) was served with 425 ml of water / noncaloric beverage and 40 g of cucumber.

<sup>3</sup>Rye bread (Jalkiuunleipä, Oululainen Ltd.) was served with 430 ml of water / noncaloric beverage and 40 g of cucumber.

<sup>4</sup>Oatmeal porridge prepared with water (Elovena, Raisio Group Ltd.) was served with 100 ml of water / non-caloric beverage.

<sup>5</sup>Instant mashed potato prepared with water (Idahoan Foods Ltd) was served with 170 ml of water and 40 g of cucumber.

<sup>6</sup>Filtered coffee beverage 125 ml (7 g ground coffee, Juhlamokka; Gustav Paulig Ltd.) was served with 375 ml of water. The amount of caffeine was 151 mg.

<sup>7</sup>Filtered coffee beverage 250 ml (14 g ground coffee, Juhlamokka; Gustav Paulig Ltd.) was served with 250 ml of water. The amount of caffeine was 303 mg.

<sup>8</sup>All test meals contained mashed potato (Van Gogh, prepared with water and margarine). The portion size of the mashed potato was 362 g in all mashed potato-based meals, except in the meal containing rye bread, which was served with 170 ml of water and 40 g of cucumber.

<sup>9</sup>Rapeseed oil (Kultasula Rypsiöljy, Raisio Ltd.) 30 g blended with mashed potato was served with 170 ml of water and 40 g of cucumber.

<sup>10</sup>Chicken breast (HK Ruokatalo Ltd) 108 g was served with 100 ml of water and 40 g of cucumber.

<sup>11</sup>The salad contained cucumber, tomato, and lettuce. The portion size of the salad was 120 g, and it was served with 50 ml of water.

<sup>12</sup>Rapeseed oil 30 g + Chicken breast 108 g + Salad 120 g and was served with 50 ml of water.

<sup>13</sup>The portion size of mashed potato was 272 g + Rapeseed oil 30 g + Chicken breast 108 g + Salad 120 g + Rye bread (whole-grain rye flour 36%, Vaasan Ruispalat, Vaasan&Vaasan Ltd.) 30g, margarine 80% fat (Valio Ltd., Finland) 6 g was served with 90 ml of water.

<sup>14</sup>Glucose solution (25 g of available carbohydrates) mixed with 21 g of alcohol (Spiritus Fortis A, Ethanolum, 96%, Berner Ltd.) was served with 250 ml of water.

<sup>15</sup>Nikolai Lager, 0.0%vol (Sinebrychoff Ltd.) 640 ml.

<sup>16</sup>Nikolai Lager, 4.5%vol (Sinebrychoff Ltd.) 510 ml containing 21 g of alcohol.

<sup>17</sup>Sugars + malto-oligosaccharides.

#### 4.3.5 Low- and high-GI meals

In *Study III*, two test meals with different GI values (33 for low-GI meal and 81 for high-GI meal), but similar macronutrient content were evaluated. The GI values of the meals were calculated using the recommended method (FAO/WHO 1998), and the GI values of each component of the meals were based on the GI database of the National Institute for Health and Welfare (Similä et al. 2009). The glucose solution, used as a reference, was tested twice. The test meals and the reference meal contained 50 g of available carbohydrates. Both test meals contained the same amounts of energy, protein, fat, and fibre. The energy supply from macronutrients was in accordance with the Nordic Nutrition Recommendation (Nordic Council of Ministers 2004); 55% energy as carbohydrate, 15% energy as protein, and 30% energy as fat.

Both meals were served with a 150-ml beverage of choice, either water, coffee, or tea. Water was chosen by 12 (3 normal weight and NGT, 4 overweight and NGT, 2 normal weight and IGT, and 3 overweight and IGT), coffee by 27 (8 normal weight and NGT, 5 overweight and NGT, 6 normal weight and IGT and 8 overweight and IGT), and tea by 9 (1 normal-weight and NGT, 3 overweight and NGT, 4 normal weight and IGT, and 1 overweight and IGT) subjects.

The nutrient composition of the test meals was analysed by AnalyCen Laboratory (Lidköping, Sweden). The protein content of the meals was estimated by the method of Kjeldahl (Eagan et al. 1981), and the fat content by a modified method of Schmid–Bondzynski–Ratzlaff (Croon and Fuchs 1980). Free sugars (glucose, fructose, lactose, maltose, and sucrose) were determined by the Dionex ion chromatograph system, and the starch contents of the test meals were analysed by the modified Åman and Hesselman method (1984). Total fibre was analysed by an enzymatic gravimetric procedure (Association of Official Analytical Chemists 45.4.07). The study meals and their nutrient compositions are shown in Table 11.

**Table 11.** Nutrient composition of the study meals in *Study III*.

	Weight	Energy	ACHO <sup>1</sup>	Protein	Fat	Fibre	Given GI	Overall GI of the meal <sup>2</sup>
	g	kJ/portion	g/portion	g/portion	g/portion	g/portion		
<b>Reference food</b>	50	850	50.0					100
<b>LOW-GI meal<sup>2</sup></b>	420.5	1527	50.2	14.0	12.1	7.2		33
Whole-grain rye bread <sup>3</sup>	30		10.6				50	
Barley porridge prepared with lactose-free milk <sup>4</sup>	160		20.3				35	
Home-made raspberry juice sweetened by 17 g of fructose	170		19.4				23	
Cucumber	40		0.6				39	
Margarine (7.5 g) and cheese (13 g) <sup>5</sup>	21.5						-	
<b>HIGH-GI meal<sup>2</sup></b>	406.5	1523	50.0	14.2	11.9	7.1		81
Wheat baguette <sup>6</sup>	36		20.8				79	
Wheat porridge prepared with water <sup>7</sup>	160		14.1				76	
Rye fibre <sup>8</sup>	5		1.3				40	
Home-made raspberry juice sweetened by 13.5 g of glucose	135		15.4				93	
Cucumber	40		0.6				39	
Margarine (6.5 g) and cheese (24 g) <sup>5</sup>	30.5						-	



<sup>1</sup>ACHO, Available carbohydrates

<sup>2</sup>Overall GIs of the test meals were estimated by using the equation  $GI = GI_A \times g_A \div g_{tot} + GI_B \times g_B \div g_{tot}$ , where  $GI_A$  is the GI of component A,  $g_A$  is the amount of available carbohydrate in component A (g), and  $g_{tot}$  is the total amount of available carbohydrates measured in grams in the test meal.

<sup>3</sup>Meals were served with 150 ml of filtered coffee (Juhlamokka, Gustav Paulig Ltd.) / instant tea (Lipton Yellow Label; Unilever Ltd.) /water, and with 30 ml of water.

<sup>4</sup>REAL-ruisleipä (Fazer Ltd.)

<sup>5</sup>Barley porridge (Ohrasuurimo, Myllyn Paras Ltd.) prepared with lactose-free milk (1.5% fat) (Laktoositon kevytmaitojuoma, Valio Ltd.)

<sup>6</sup>Flora margarine 70% (Unilever Ltd.) and Edam cheese 24% (Valio Ltd.)

<sup>7</sup>Vehnäpatonki (Fazer Ltd.)

<sup>8</sup>Wheat porridge prepared with water (Vehnähutale, Nalle, Raisio Ltd.)

## 4.4 Blood sampling and laboratory methods

The analyses of glucose and insulin were performed at the National Institute for Health and Welfare.

### 4.4.1 Capillary blood glucose

In all studies, capillary blood glucose was analysed directly by using a glucose meter (HemoCue® Glucose 201 meter, HemoCue Ltd., Espoo, Finland). Before capillary blood sampling from the finger-tip, the subjects warmed their hands under running warm water to increase peripheral blood flow and facilitate blood sampling. The capillary blood sample was taken using a lancet (Medlance® Red, HTL-STREFA S.A., Poland) with a drop of blood collected into a HemoCue cuvette, and blood glucose concentration was measured using a HemoCue 201 Analyser. The HemoCue Glucose system is based on a glucose dehydrogenase method. The results were automatically transformed to express the plasma glucose values. A quality-control solution recommended by HemoCue was measured twice every study morning; the CV of these measurements was 1.1% (*Study I*), 1.5% (*Study II*), 1.2% (*Study III*), 0.3% (*Study IV*), and 0.6% (*Study V*).

### 4.4.2 Venous blood glucose

In *Study I*, an intravenous cannula was inserted into a vein in the antecubital fossa, and blood samples (5 ml/sample) were drawn for venous blood glucose determination. The samples were collected in a fluoride-citrate tube (Venosafe®, Terumo Sweden Ltd., Västra Frölunda, Sweden) and were kept in the refrigerator (4°C) for 20 min until centrifugation. The samples were centrifuged for 15 min at  $4000 \times g$  at 20°C to separate the plasma. Plasma glucose was analysed by a hexokinase method (Thermo Electron Ltd., Vantaa, Finland). The inter-assay and intra-assay coefficients of variation for venous glucose determination were 3.4% and 1.1%, respectively.

### 4.4.3 Capillary blood insulin

Finger-prick capillary blood samples (0.5 ml/sample) were drawn for insulin determination. The samples were collected in non-heparin-treated gel tubes (Capiject®, Terumo Sweden Ltd., Västra Frölunda, Sweden). The samples were allowed to clot for 20 min at room temperature. After clotting, the samples were centrifuged ( $4000 \times g$ ; 15 min, Rotofix 32, Hettich Zentrifugen) within 20 min, and then they were separated into serum and kept at -70°C until analysis. The serum insulin from the capillary samples was determined by an AxSYM system, which is

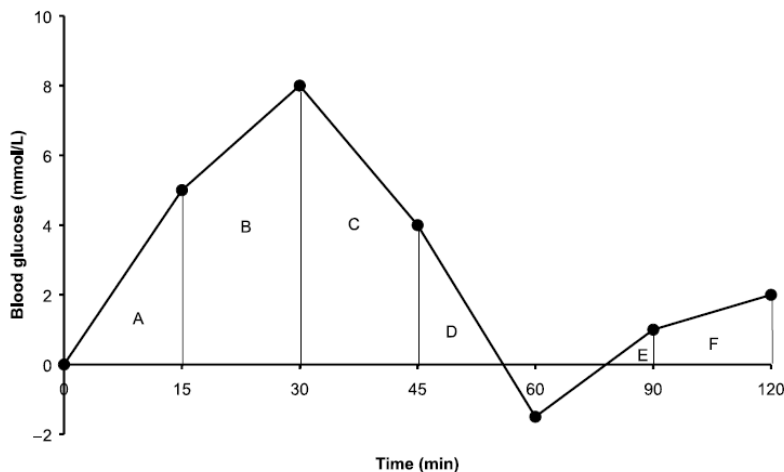
based on Microparticle Enzyme Immunoassay (MEIA) technology (Abbot Laboratories, Abbot Park, IL, USA). In *Study II*, the inter-assay coefficient of the variation (CV) of the insulin was 6.7%, in *Study III* was 6.8%, in *Study IV* was 4.8%, and in *Study V* was 1.8%.

## 4.5 Calculations and statistical analysis

Subjects' BMI was calculated as follows: the weight in kilograms divided by the square of the height in metres. Variation within subjects was expressed as the average coefficient of variation (CV), and it was calculated as follows:  $CV = 100 \times SD/\text{mean}$ .

### 4.5.1 Incremental area under the curve (IAUC)

For each test, incremental areas under the glucose and insulin response curves (IAUCs), ignoring any area under the baseline value, were calculated with the trapezoid method (WHO/FAO 1998). The method is illustrated in Figure 1.



**Figure 1.** Incremental area under the curve (IAUC) equals the sum of the areas A to F. The area under the baseline (negative area) is not included. The figure is adapted from Arvidsson-Lenner et al. (2004).

There were five missing glucose values (one venous value for rye bread, one capillary value and one venous value for both glucose solution and white bread) in *Study I*. Estimates for missing values were calculated using the corresponding average glucose value of the other subjects (rye bread) or the mean of the subject's

other two reference tests (glucose solution and white bread). Each estimate was corrected by the difference between the level of the incomplete curve and the mean level of the complete curves (the levels were estimated as the mean of the blood glucose of all time-points, except the missing point). No values were estimated, nor were any IAUCs calculated for two white bread tests with more than one missing venous sample. The IAUCs for 180 min were missing for three test visits due to missing capillary and venous samples at 180 min. The other studies had no missing capillary blood values.

#### 4.5.2 Glycaemic index (GI)

The glycaemic index (GI) was defined as the percentage of the plasma glucose IAUC in study meal of that in the reference food (glucose solution or white bread)(WHO/FAO 1998).

$$\text{GI} = \text{IAUC (study meal)} \div \text{mean IAUC (reference)} \times 100$$

#### 4.5.3 Insulinaemic index (II)

The insulinaemic index (II) was defined as the percentage of the serum insulin IAUC in the study meal of that in the reference glucose solution.

$$\text{II} = \text{IAUC (study meal)} \div \text{mean IAUC (reference)} \times 100$$

In *Studies II-V*, the 2-h insulin curves that included one or more strongly or three or more mildly haemolysed serum samples were excluded from the analyses. In *Study II*, one 2-h insulin curve for a glucose solution with small coffee and five insulin curves for a glucose solution were excluded due to haemolysis. In *Study III*, two insulin curves for low-GI meal were excluded; both excluded insulin curves were among overweight subjects with NGT. In *Study IV*, 32 insulin curves were excluded from the analysis, 14 for a glucose solution, 6 for mashed-potato, 4 for mashed potato with rapeseed oil, 3 for mashed potato with chicken breast, and 5 for all meals that contained salad. In *Study V*, two insulin curves, one for a glucose solution and one for beer, were excluded.

#### 4.5.4 GI of the meal

The overall GIs ( $GI_{pred}$ ) of the test meals were calculated by using the recommended method of weighting the GI of each component in the test meal (WHO/FAO 1998).

$$GI_{pred} = GI_A \times g_A \div g_{tot} + GI_B \times g_B \div g_{tot} \dots,$$

where  $GI_A$  is the GI of component A,  $g_A$  is the amount of available carbohydrate in component A (g), and  $g_{tot}$  is the total amount of available carbohydrates measured in grams in the test meal.

In *Study III*, the GI values of each component of the meals were derived from the GI database of the National Institute for Health and Welfare (Similä et al. 2009).

#### 4.5.5 Statistical analysis

All results are presented as means and standard deviations (SDs). Differences in means were considered significant at  $P < 0.05$ .

In *Study I*, to determine the effect of using a single test versus a double or a triple test of the reference food on GI values, one or two IAUCs of the reference food were selected randomly from the three IAUCs of reference foods for each subject. The GI values were then calculated using the IAUC for the randomly selected glucose or white bread tests. All statistical analyses were carried out using SAS software (version 8.2, SAS Institute Inc., Cary, NC, USA).

In *Study III*, the independent sample t test with Bonferroni corrections was used for testing the differences between study groups. Insulin responses were non-normally distributed; statistical significance was therefore assessed by using the non-parametric Wilcoxon test. All statistical analyses were conducted using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA).

In *Studies II, IV, and V*, the incremental peaks of glucose and insulin, the IAUCs, and GI and II values were analysed by non-parametric Friedman's test for repeated measures comparisons. Post-hoc comparisons were performed, adjusting for multiple comparisons with Bonferroni correction if Friedman's tests showed significant effects. A non-parametric Wilcoxon signed-rank test with Bonferroni corrections was used when the differences between beverages were tested. In *Studies II and V*, all statistical analyses were carried out using SAS software (version 8.2, SAS Institute Inc., Cary, NC, USA), and in *Study IV* using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA).



# 5 Results

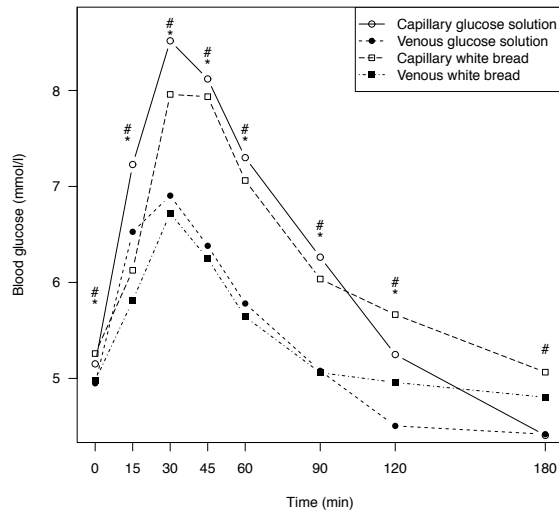
## 5.1 Methodological choices

### 5.1.1 Blood sampling

In *Study I*, two blood sampling methods, capillary and venous, were compared. The targeting preference was in capillary blood samples, which were taken as precisely as possible at 15, 30, 45, 60, 90, 120, and 180 min after the start of the meal. The venous samples were taken after the capillary samples, the average delays being 3.0-3.7 min at different time-points, but the differences between the venous IAUCs based on real and fixed time were not statistically significant (data not shown). However, the CVs of the IAUCs were slightly lower when real time was used, and thus, we used real time when comparing capillary and venous sampling.

Capillary blood samples elicited higher postprandial glucose responses than did venous blood samples (Figure 2). The 2-h glycaemic IAUCs measured from capillary samples were significantly larger, almost twice those measured from venous samples (Table 12). The CVs for capillary IAUCs were 20-40% lower than those for venous IAUCs (Table 12).

Contrary to the IAUCs, capillary samples produced smaller GI values than venous samples (Table 13). In addition, capillary samples had clearly lower variation, resulting in smaller CVs than venous samples (with the exception of rye bread when the reference food was glucose solution). Although the CV diminished slightly when three reference tests were used, capillary GIs were similar with two and three reference tests (Table 13).



**Figure 2.** Mean fasting and postprandial capillary and venous blood glucose after consumption of glucose solution and white bread. The values at the different time-points were based on 33 blood samplings. For glucose solution, the glucose concentration differed significantly between capillary and venous samples in all time-points, excluding 180 min. For white bread, the glucose concentration differed significantly between capillary and venous samples at all time-points. \* = significant difference between capillary and venous glucose solution. # = significant difference between capillary and venous white bread.



**Table 12.** Incremental areas under the curves for reference foods.<sup>1</sup>

	Glucose solution				White bread			
	Capillary blood		Venous blood		Capillary blood		Venous blood	
	mean±SD	CV%	mean±SD	CV%	mean±SD	CV%	mean±SD	P <sup>2</sup>
IAUC of 3 tests	228±84	36	120±61	51	171±47	28	92±32	35 <0.001
IAUC of 2 tests	230±80	35	126±66	52	183±52	28	100±49	49 <0.001
Absolute percentage of difference <sup>3</sup>	7±5		11±7		10±7		23±16	
IAUC of 1 test	220±98	45	117±86	73	186±66	36	101±59	58 0.001
Absolute percentage of difference	16±12		31±25		23±16		32±27	
P <sup>4</sup>	0.68		0.49		0.13		0.60	

<sup>1</sup>n=11 (except for venous IAUCs of white bread, n=9). The incremental area under the curve (IAUC) was calculated by using the trapezoidal rule.  
<sup>2</sup>A paired t test was used to test the difference between capillary and venous IAUCs (11 pairs for glucose solution and 9 pairs for white bread).  
<sup>3</sup>Absolute percentage of difference of the IAUC from the IAUC based on three reference tests.  
<sup>4</sup>A repeated-measures ANOVA was used to test differences between IAUCs of 1, 2, and 3 reference tests.

**Table 13.** Glycaemic indices for rye bread, oatmeal porridge, and instant mashed potato.<sup>1</sup>

	Number of tests	Glucose solution				White bread			
		Capillary blood		Venous blood		Capillary blood		Venous blood	
		Mean±SD	CV%	Mean±SD	CV%	Mean±SD	CV%	Mean ± SD	CV%
Rye bread	1	85±39	45	153±183	120	99±46	46	144±119	83
	2	78±38	48	77±16	21	96±37	39	164±197	121
	3	77±31	40	82±18	22	102±40	39	128±80	63
<i>P</i>		0.48		0.31		0.15		0.68	
Oatmeal porridge	1	82±38	46	221±362	164	97±51	53	153±141	92
	2	74±26	35	91±37	40	92±35	38	177±232	131
	3	74±23	31	95±41	43	98±36	36	134±97	73
<i>P</i>		0.48		0.12		0.44		0.75	
Instant mashed potato	1	90±40	44	182±247	135	108±58	53	148±117	79
	2	79±21	27	86±35	41	102±42	41	164±186	113
	3	80±21	26	92±40	44	108±45	41	134±84	63
<i>P</i>		0.42		0.31		0.18		0.75	

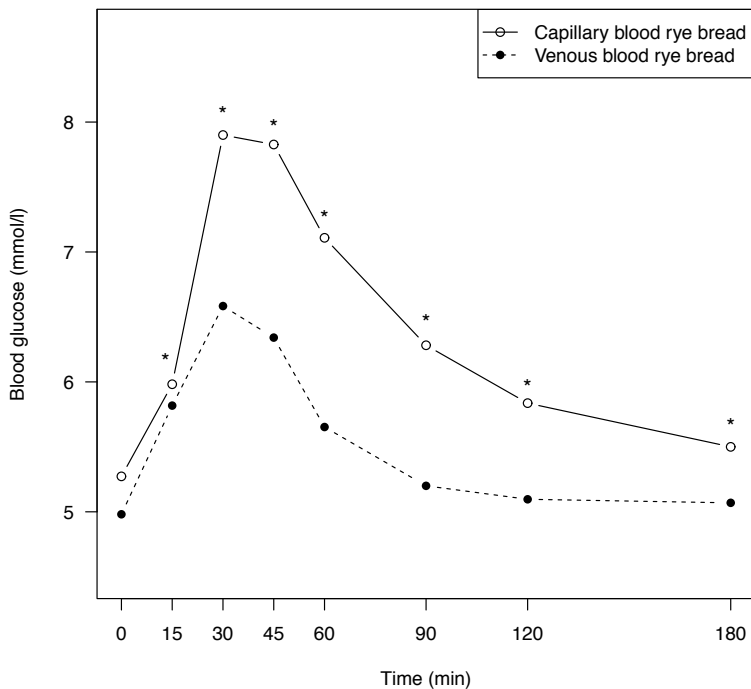
<sup>1</sup>  $r=11$  (except for venous samples of white bread,  $n=9$ ). The glycaemic index (GI) was defined as the percentage of the glucose IAUC of the test food divided by that of the reference glucose solution/white bread. A repeated-measures ANOVA was used to test differences between GIs of 1, 2, and 3 reference tests.

The recommended method of calculating IAUCs is based on seven blood samples. The GI values based on four blood samples at the time-points 0, 30, 60, and 120 min resulted in similar GI values and CVs (Table 14). Extending the blood sampling to 180 min tended to produce slightly higher GI and CV for rye bread than the recommended 120-min blood sampling. This was mainly due to the glucose level at 180 min not reaching the baseline level (Figure 3).

**Table 14.** Glycaemic indices for rye bread, oatmeal porridge, and instant mashed potato based on capillary blood measurements at 0, 15, 30, 45, 60, 90, and 120 min (ordinary), with an additional sample taken at 180 min (extended), or at 0, 30, 60, and 120 min (reduced) using glucose solution as a reference.<sup>1</sup>

	Rye bread		Oatmeal porridge		Instant mashed potato	
	mean±SD	CV%	mean±SD	CV%	mean±SD	CV%
Ordinary	78±33	42	76±23	30	79±22	28
Extended	84±42	50	77±25	32	79±22	31
Reduced	86±40	47	78±30	38	79±28	35
<i>P</i> <sup>2</sup>	0.07		0.73		0.90	

<sup>1</sup> *n*=10. The glycaemic index (GI) was defined as the percentage of the glucose IAUC of the test food divided by that of the reference glucose solution. <sup>2</sup> A repeated-measures ANOVA was used to test the differences between GIs.



**Figure 3.** Changes in blood glucose after the consumption of rye bread. The glucose concentration differed significantly between capillary and venous samples at all time-points, excluding 0 min. \* = significant difference between capillary and venous blood samples.

### 5.1.2 Reference food

In *Study I*, two different reference foods, glucose solution and white bread, were compared. Both reference foods were repeated three times. The glucose solution elicited a more rapid initial rise and a higher peak rise at 30 min than white bread (Figure 2). The glucose solution produced 1.3 times higher IAUCs than white bread when the reference foods were tested two and/or three times. The CVs of the white bread were, however, lower than those of the glucose solution (Table 12).

When white bread was used as the reference food, both the GI values and their variation were higher. However, the CVs diminished when the reference food was tested two or three times. White bread provided ~1.3 times higher GIs than glucose solution when the GIs were measured from capillary samples (Table 13).

When the GI values were measured from capillary blood samples and glucose solution was used as the reference, rye bread resulted in a GI of  $77 \pm 31$ , oatmeal porridge  $74 \pm 23$ , and instant mashed potato  $80 \pm 21$  (Table 13). The CVs were the

lowest when capillary blood was used and the reference glucose solution was tested three times, excluding rye bread, which produced the lowest CV when venous blood was used and the reference was repeated twice (Table 13). When white bread was tested as a test food and it was tested once and the reference glucose solution was tested three times, white bread resulted in a GI of  $89\pm38$  (Table 15).

### 5.1.3 Number of trials

Reference foods tested two or three times produced similar IAUCs than when tested only once. The variation decreased when the reference food test was repeated, but repeating the test twice did not lead to more benefit, and the CVs for once or twice repeated reference tests were similar. The absolute percentage differences were slightly lower for glucose solution than for white bread, but the difference was significant only for the venous sample tested twice (Table 12).

The capillary GIs were lower with two or three tests of the reference food than with one test. The CVs were also lower when two or three tests were used than when the reference was tested once. The CVs of the GI values (glucose reference tested three times and capillary blood sampling) for rye bread, oatmeal porridge, and instant mashed potato were 40%, 31%, and 26%, respectively (Table 13).

Because the 2-h responses to glucose solution and white bread were tested three times, it is possible to estimate the effect of trial numbers of the test food on the GI values and the variation of the GI values. When white bread (the test food) was tested twice and glucose solution (the reference food) was tested two or three times, the GI value of white bread diminished from 89 (white bread tested once) to 84 and 85, respectively, and the variation clearly decreased (Table 15). When both glucose solution and white bread were tested three times, the GI of white bread decreased further to 79 (Table 15).

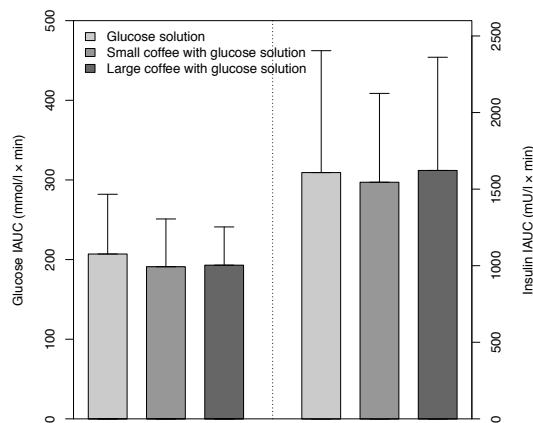
**Table 15.** Glycaemic indices based on capillary blood measurements for white bread using glucose solution as a reference.<sup>1</sup>

		Glucose solution					
		Tested once		Tested twice		Tested three times	
		Mean±SD	CV%	Mean±SD	CV%	Mean±SD	CV%
<b>White bread</b>	Tested once	94±37	40	89±41	46	89±38	43
	Tested twice			84±23	27	85±21	24
	Tested three times					79±19	24

<sup>1</sup>  $n=11$ . The glycaemic index (GI) was defined as the percentage of the glucose IAUC of the test food divided by that of the reference glucose solution/white bread.

#### 5.1.4 Coffee as the beverage of the meal

*Study II* tested two different portions of coffee, 125 ml with 150 mg of caffeine, and 250 ml with 300 mg of caffeine, served with a glucose solution containing 50 g of available carbohydrate. The coffee portions with glucose solution produced similar glucose responses, resulting in IAUCs of  $191 \pm 60$  and  $193 \pm 48$ , respectively (Figure 4). The reference glucose alone produced an IAUC of  $207 \pm 75$ . The coffee portions with glucose solution yielded similar insulin IAUCs ( $1545 \pm 580$  for small coffee with glucose solution,  $1622 \pm 755$  for large coffee with glucose solution) as that of pure glucose solution ( $1855 \pm 797$ ) (Figure 4). The coffee portions with glucose solution produced GIs of 104 and 103, respectively, and IIs of 89 and 92, respectively. No significant differences between the reference glucose solution and the coffee portions with the glucose solution were found in the IAUCs or in the indices.

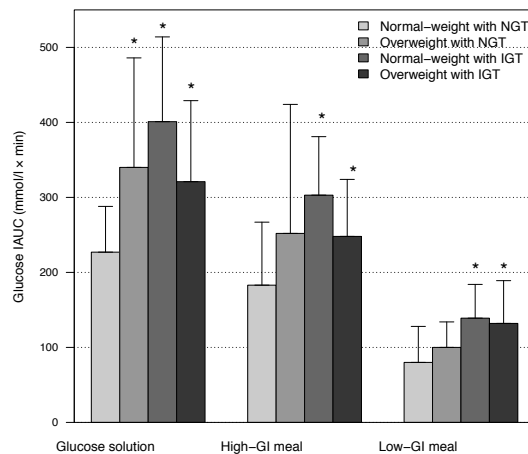


**Figure 4.** Glucose and insulin IAUCs of the reference glucose solution, the glucose solution with small coffee, and the glucose solution with large coffee (Means±SDs). No significant differences were present between the glucose IAUCs or between the insulin IAUCs.

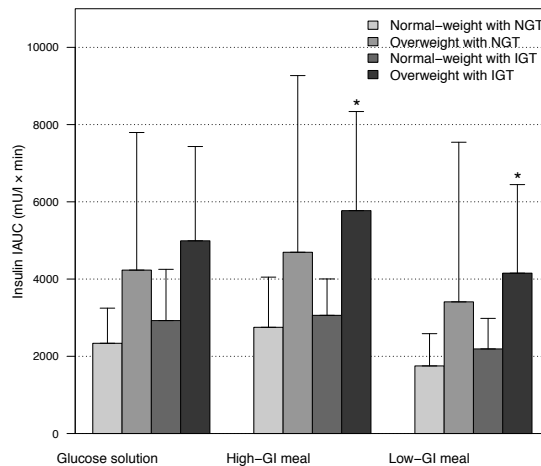
## 5.2 Subjects' characteristics

### 5.2.1 Glucose tolerance

In *Study III*, two isoenergetic breakfast meals were compared that had the same macronutrient composition but different GI values (predicted GI value of 81 for the high-GI meal and 33 for the low-GI meal) in subjects with different physiological background. The glucose responses were 66% and 74% larger and insulin responses were 11% and 28% larger for the high- and low-GI meals, respectively, in normal-weight subject with IGT relative to normal-weight subjects with NGT (Figures 5 and 6). The differences were significant for the glucose response ( $P < 0.01$  for both meals), but not for the insulin response.



**Figure 5.** Glucose IAUCs of the glucose reference solution, the high-GI meal, and the low-GI meal ( $n=12$ ). \* = Significantly different from the glucose IAUC of normal-weight subjects with NGT.



**Figure 6.** Insulin IAUcs of the glucose reference solution, the high-GI meal, and the low-GI meal (n=12, except n=10 for the low-GI meal in overweight subjects with NGT). \* = Significantly different from the insulin IAUc of normal weight-subjects with NGT.

The high-GI meal resulted in a GI value of  $79 \pm 16$  and an II value of  $116 \pm 37$ , respectively, and the low-GI meal produced a GI value of  $34 \pm 16$  and an II value of  $74 \pm 25$  in normal-weight subjects with NGT. Both the GI and II values of the low-GI meal were significantly different from the values of the high-GI meal ( $P < 0.001$ ). The GI and II values were essentially the same in normal-weight subjects with IGT (GIs  $80 \pm 22$  and  $36 \pm 11$ , and IIs  $114 \pm 33$  and  $80 \pm 22$ ) for the high- and low-GI meals. Neither GI values nor II values differed significantly between the groups.

### 5.2.2 Body weight

Glucose responses were 38% and 25% larger and insulin responses 70% and 114% larger for the high- and low-GI meals, respectively, in overweight subjects with NGT ( $252 \pm 172$  and  $4694 \pm 4575$  for high-GI meal,  $100 \pm 34$  and  $3410 \pm 4133$  for low-GI meal) than in normal-weight subjects with NGT ( $183 \pm 76$  and  $2751 \pm 1299$  for high-GI meal,  $80 \pm 48$  and  $1715 \pm 835$  for low-GI meal). However, these differences did not reach statistical significance (Figures 5 and 6). Overweight was associated



with larger variation in both glucose and insulin responses than normal weight, except for the glucose response to the low-GI meal.

The measured GI value of the low-GI meal was  $32 \pm 15$  in overweight subjects with NGT compared with  $34 \pm 16$  in normal-weight subjects with NGT ( $P=0.17$ ). The corresponding GI values for the high-GI meal were  $69 \pm 18$  and  $79 \pm 16$  ( $P=0.12$ ). The II value of the low-GI meal was 74, in both normal-weight and overweight subjects with NGT. The corresponding II values for the high-GI meal were  $116 \pm 27$  and  $104 \pm 26$  ( $P=n.s.$ ).

When overweight and impaired glucose tolerance manifested together in the same subject, 2-h glucose IAUC to the high-GI meal was virtually identical to that observed in overweight subjects with NGT ( $248 \pm 76$  vs.  $252 \pm 172$ ,  $P=n.s.$ ). In addition, the 2-h glucose IAUC to the low-GI meal was similar in normal-weight subjects with IGT and overweight subjects with IGT ( $139 \pm 45$  vs.  $132 \pm 57$ ,  $P=n.s.$ ). The GI values of the meals were  $41 \pm 9$  for the low-GI meal and  $81 \pm 32$  for the high-GI meal in overweight subjects with IGT. No statistically significant differences were found relative to normal-weight subjects with NGT.

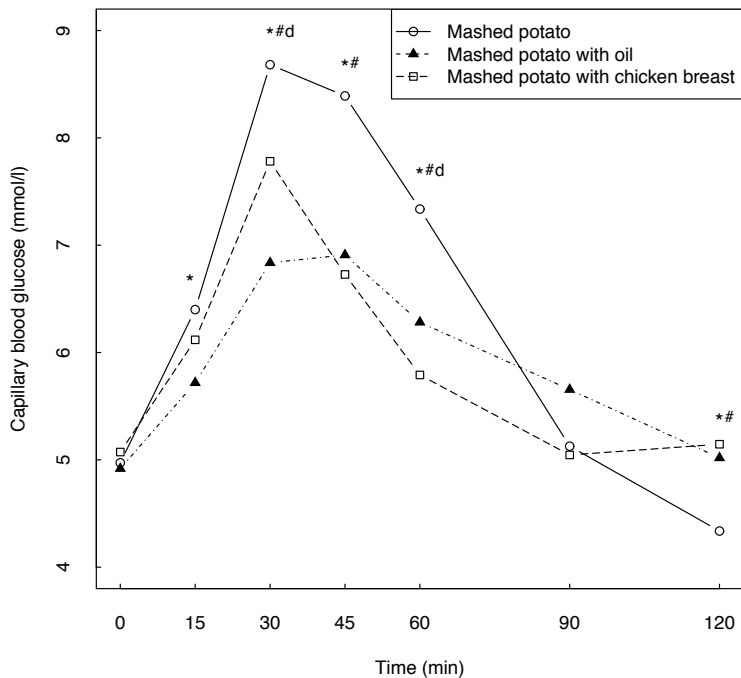
Overweight increased insulin responses to the meals. The insulin IAUC in overweight subjects with NGT was  $4694 \pm 4575$  for the high-GI meal and  $3410 \pm 4133$  for the low-GI meal, and in overweight subjects with IGT the corresponding figures were  $5772 \pm 2566$  for the high-GI meal and  $4153 \pm 2293$  (Figure 6). The insulin responses did not differ significantly between groups. The II values of overweight subjects with IGT were  $121 \pm 38$  for the high-GI meal and  $82 \pm 13$  for the low-GI meal. The corresponding II values of normal-weight subjects with NGT were similar,  $114 \pm 33$  and  $80 \pm 22$ .

## 5.3 Macronutrients

### 5.3.1 Fat

In *Study IV*, mashed potato alone produced the largest 2-h glucose response, with an IAUC of  $197 \pm 94$ , compared with the other mashed potato-based meals. When mashed potato was ingested with 30 g of rapeseed oil, the initial glucose increase slowed down, resulting in a ~30% smaller IAUC,  $136 \pm 74$  (Figures 7 and 8), relative to the mashed potato meal. The oil addition also delayed the decrease in blood glucose concentration. The mashed potato alone produced a GI value of  $108 \pm 48$  and the mashed potato with oil a GI of  $71 \pm 32$ . Although the decrease was evident, it did not reach statistical significance due to large variation.

The insulin IAUC of the mashed potato was  $2240 \pm 2000$ . The addition of oil also decreased insulin response, resulting in an IAUC of  $1350 \pm 450$  (Figure 8). The mashed potato alone produced an II of  $118 \pm 40$  and the mashed potato with oil an II of  $97 \pm 46$ . No significant differences emerged between insulin responses.

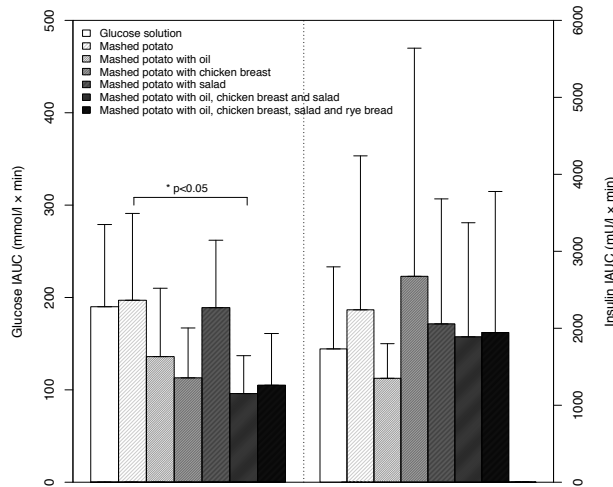


**Figure 7.** Mean changes in capillary blood glucose in healthy subjects after the consumption of mashed potato-based meals. \* = significant difference between mashed potato and mashed potato with oil, # = significant difference between mashed potato and mashed potato with chicken breast, d = significant difference between mashed potato with oil and mashed potato with chicken breast.

### 5.3.2 Protein

In *Study IV*, the addition of a protein source to a mashed potato meal, i.e. chicken breast containing 30 g of protein, provided a 42% reduction in glucose IAUC, from  $197 \pm 94$  to  $113 \pm 54$  ( $P=0.08$ ) (Figure 8). When chicken breast (containing 30 g of protein), rapeseed oil (30 g), and salad (120 g) were added to a mashed potato meal, the glucose response diminished further to  $96 \pm 41$  ( $P<0.05$ ). When part of the available carbohydrates of mashed potato was substituted with that of rye bread, the glucose IAUC was moderately increased, from  $96 \pm 41$  to  $105 \pm 56$  (Figure 8). The ingestion of protein with mashed potato decreased the GI value of mashed potato from  $108 \pm 48$  to  $64 \pm 33$  ( $P=0.05$ ). When the mashed potato meal contained oil, chicken breast, and salad, the GI value decreased further to  $54 \pm 21$  ( $P=0.03$ ), but when the meal also contained rye bread the GI slightly increased to  $65 \pm 38$ .

The mashed potato with chicken breast provided a 16% increase in the insulin IAUC relative to the insulin IAUC of mashed potato alone ( $2675 \pm 2964$  vs.  $2240 \pm 2000$ ). Mashed potato alone produced an II of  $118 \pm 40$  and with chicken breast an II of  $148 \pm 78$ . No significant differences emerged between insulin responses.



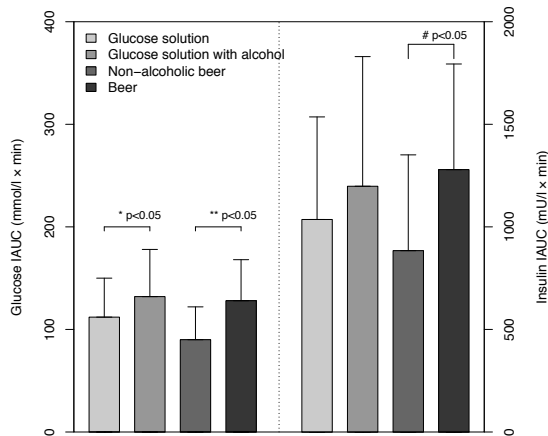
**Figure 8.** Glucose and insulin IAUCs of the reference glucose solution and mashed potato-based meals (Means+SDs). \* = The glucose IAUC of mashed potato with oil, chicken breast, and salad differed significantly ( $P < 0.05$ ) from the glucose IAUC of mashed potato alone.

### 5.3.3 Alcohol

In *Study V*, the glucose solution with 21 g of alcohol resulted in 18% glucose (IAUC  $132 \pm 46$ ,  $P = 0.03$ ) and similar insulin (IAUC  $1198 \pm 632$ ,  $P = 0.48$ ) 2-h response IAUCs as the reference glucose solution (IAUCs of glucose and insulin were  $112 \pm 38$  and  $1036 \pm 500$ , respectively). Glucose solution with alcohol produced a GI of 119 and an II of 121. No significant differences were found between the reference glucose solution and the glucose solution with alcohol in GI and II values.

Compared with the reference glucose solution, beer produced similar glucose ( $128 \pm 40$ ,  $P = 0.58$ ) and insulin ( $1231 \pm 522$ ,  $P = 0.12$ ) IAUCs (Figure 9). Non-alcoholic beer produced 20% lower glucose IAUC ( $90 \pm 32$ ,  $P = 0.06$ ) and similar insulin IAUC ( $884 \pm 467$ ,  $P = 0.58$ ) as the reference glucose solution, with a GI of 80

and an II of 88. Comparing the differences between the beers, regular beer yielded 42% higher glucose ( $P=0.04$ ) and 39% higher insulin ( $P=0.07$ ) IAUCs than the non-alcoholic beer. Beer produced a GI value of 119, and an II value of 131. The GI and II values of beer and non-alcoholic beer were significantly different ( $P=0.02$  and  $P=0.02$ , respectively). Glucose solution with alcohol and the beer produced similar glucose and insulin IAUCs (Figure 9).



**Figure 9.** Mean glucose and insulin IAUCs of the reference glucose solution, glucose solution with alcohol, non-alcoholic beer, and regular beer (Means+SDs). \* = Significantly different from the reference glucose solution ( $P<0.05$ ). \*\* = Significantly different from the non-alcoholic beer ( $P<0.05$ ). # = Significantly different from the non-alcoholic beer ( $P<0.05$ ).

# 6 Discussion

The prevalence of type 2 diabetes is rapidly increasing worldwide, necessitating modifications in diet and lifestyle. Dietary advice for prevention of chronic diseases has recently undergone a notable change. Replacing fat, especially saturated fat, with carbohydrates has been reported not to be effective (Astrup et al. 2011, Jakobsen et al. 2009). Consequently, the quality of carbohydrates has become increasingly important (Buyken et al. 2014, Overby et al. 2013). For decades, glycaemic index (GI) and its impact on health have been subjects of considerable interest in the scientific community as well as in the general public. There are some misunderstandings of GI and its association with nutritional values of foods. For example, ripeness of fruit has an impact on GI value, but it is unimportant for nutritional composition (Aston et al. 2010). Different types of rice have very different GI values, but at the same time highly similar nutrient compositions (Aston et al. 2010). Concern has been raised that excluding some starchy foods with high GI values, e.g. whole-grain breads or potatoes, may have a negative impact on micronutrient intake. A recent study established that consuming foods with low GI values may help in reaching the nutrition recommendations goals (Louie et al. 2012).

Several methodological aspects influence measured GI values. This thesis considers some of these, e.g. the subject's physiological background, choice of reference food, method of blood sampling, number of tests performed on the reference food, and the effect of fat, protein, coffee, and alcohol on the measured outcome.

The main findings of this thesis were that capillary blood sampling should be used in determining GI values, and the reference test should be repeated at least once. Coffee can be used as a beverage in GI measurement because it does not affect GI values. We also noted that overweight or glucose tolerance does not alter measured GI values. However, macronutrients, fat and protein together, and alcohol alone can modify the GI values measured.

## 6.1 Methodological choices

### 6.1.1 Study design

In our studies, all subjects were screened with a standardized method to ensure normal glucose tolerance (WHO 1999), except in *Study III*, where two study groups included subjects with impaired glucose tolerance. Despite pre-study screening, marked individual differences can occur for various reasons, e.g. degree of insulin

sensitivity, body composition, and genetic factors. Subjects with an outlying result ( $>2$  SD from the mean) have been suggested to be removed from the GI analysis (ISO 26642:2010, 2010, Wolever et al. 1991). Previously, no general rule has existed in statistical analysis to exclude outliers from the calculation of GIs, but several reasons have been given for unrepresentative results, e.g. incorrect subject preparation, analytical error, or errors in data calculations (Brouns et al. 2005). We did not find any obvious reasons for outlying results, and even if we excluded values  $>2$  SD from the mean, the GIs did not change essentially (data not shown). Thus, without an evident reason, the outliers should not be excluded if a standardized protocol of determining GI values is used and the subjects are properly screened before enrolment in the study. Exclusion could, in fact, reduce true physiological findings. Nevertheless, the new international standard, published in 2010, recommends that if the mean within-subject CV for the reference food for the group of subjects tested is greater than 30%, one outlying result for the reference test in each subject can be deleted. However, two reference tests are still needed (International Standard ISO 26642:2010).

A common criticism of the GI is that GI values vary in different subjects or from day-to-day in the same subject (Aziz et al. 2013, Pi-Sunyer 2002). To diminish variation in glycaemic responses to study meals, we advised the subjects to control their diet and exercise on the preceding day, in accordance with the recommendations in the literature (Brouns et al. 2005). Firstly, the subjects were advised to follow their usual diet. Secondly, they were advised to consume at least 150 g of carbohydrates during the three days preceding the study day to ensure that their stored carbohydrate sources were adequate. Lastly, in *Studies I* and *IV*, the subjects were served a standardized evening meal providing 15% of the subject's daily energy, and in *Studies II, III*, and *V*, the subjects were advised to consume an evening meal that would provide 15% of the calculated daily energy requirement. To ensure maximally similar second meal effect as possible (Granfeldt et al. 2006), the major proportion of the energy of the evening meals came from carbohydrates, mainly from white bread. Because the quality of the carbohydrates of the evening meal could affect colonic fermentation and/or differences in the pattern of short-chain fatty acid (SCFA) formed, white bread was chosen as the main source of carbohydrate. Earlier studies have shown that white bread has only a moderate influence on second meal effect (Nilsson et al. 2006, Nilsson et al. 2010). Moreover, it has also been shown that providing an evening meal reduces the variation in GI values (Wolever et al. 2008). However, contradictory findings have also been presented (Campbell et al. 2003). In our postprandial studies, the subjects were not allowed to consume alcohol for 24 h before each study day. There are suggestions that alcohol consumption may have profound effects on glucose homeostasis (Shelmet et al. 1988). However, in a recent study, moderate alcohol consumption on the preceding day did not impact the glycaemic response (Godley et al. 2009).

A methodological concern with most postprandial studies testing GI is that they have included 6-12 subjects. Ten people may be insufficient to obtain reliable estimates of GI (Venn and Green 2007). This is particularly true when GI levels are high because of increased variance. According to the recent standard, a minimum of 10 subjects is acceptable (International Standard ISO 26642:2010), but larger groups would give more confidence in the estimates (Williams et al. 2008). In addition, the sample size is dependent on the level of GI. This indicates that in order to detect differences between GI values (i.e. 10 units) on the lower scale (i.e. between 30 and 40) smaller sample size is required than on the upper scale (i.e. between 70 and 80) (Venn and Green 2007). This phenomenon explains why we found only a few significant differences between GI values in our postprandial studies.

In our postprandial studies, excluding *Study III*, we had a skewed gender distribution. Originally, the aim was to recruit a more balanced gender ratio, but due to the difference in interest to participate, we ended up in with skewed gender distribution. The skewed sex ratio may have slightly affected our results because sex steroids have been shown to impact insulin sensitivity in females (Escalante Pulido and Alpizar Salazar 1999). However, no differences have been observed in glycaemic responses between males and females (Brouns et al. 2005, Wolever et al. 2008). Another limitation is that - we tested only subjects of Caucasian origin. According to some recent studies (Pratt et al. 2011, Wolever et al. 2003, Wolever et al. 2008), ethnicity has no effect on GI values, but contrary findings have also been presented (Kataoka et al. 2013, Venn et al. 2010).

### 6.1.2 Study foods and meals

The international tables of GI values contain marked variation for similar foods (Atkinson et al. 2008). One of the reasons for the variation could be the use of different food databases for calculating the sum of available carbohydrates. Commonly, use of total carbohydrate values calculated by difference ( $100 - (\text{weight in grams} [\text{protein} + \text{fat} + \text{water} + \text{ash} + \text{alcohol}])$  in 100 g of food) (FAO/WHO 2003)) increases inaccuracy (Englyst et al. 2007). In some cases, the fibre may be included in the amount of carbohydrates, along with available carbohydrates. Thus, the fact that we used a direct analysis of the starch and sugar content of the tested foods increases the validity of our measured GI and II values.

It has been recently recommended by the International Standards Organization (26642:2010) that the volume of a beverage served with a test food should be 250-500 ml. The water volume of the meal has been speculated to affect postprandial responses by the rate of gastric emptying (Torsdottir and Andersson 1989). A threefold increase in the water volume (from 200 ml to 600 ml) has resulted in a significant 21% increase in glucose response (Sievenpiper et al. 1998), but the opposite findings have also been demonstrated with the water volume varying from 50 ml to 1000 ml (Young and Wolever 1998). An increase in water volume reduces

osmolality, thus enhancing gastric emptying and leading to higher glucose responses (Thompson et al. 1982). Therefore, water volume may also have implications in glycaemic testing. On the other hand, studies determining GI values have suggested that varying the volume of a beverage does not significantly modify the results. The standardized volume of beverage should be used, but more research is needed to establish the optimal volume (Young and Wolever 1998). In all of our postprandial studies, the water volume of the meals was standardized to ~500 ml by adjustment of water, excluding the possibility that the differences in glucose responses are due to the varying volume of the meals.

Typically in GI testing, the subjects are allowed to consume coffee, tea or water with the study meals. In our postprandial studies, the subjects were not given the opportunity to choose the beverage; water was the beverage provided, with the exception of *Study I*, where three subjects chose non-caloric juice as the beverage. In addition, in *Study III*, the subjects were allowed to choose either water, coffee, or tea as the beverage consumed with meals. A total of 48 subjects (56%) chose coffee. Combining both caffeine-containing beverages, the proportion of subjects drinking coffee or tea was 75%. As seen in *Study II*, coffee modified glucose and insulin responses only modestly, and therefore, it is unlikely that caffeine-containing beverages affected the results.

### 6.1.3 Blood sampling method

In *Study I*, we observed that capillary plasma glucose levels were consistently higher than the venous levels during the first two hours for all study meals, which is in line with previous findings (Granfeldt et al. 1995, Wolever et al. 1988b). The capillary blood samples produced almost twice as large IAUCs, but the CVs of the IAUCs were, however, significantly lower than for venous blood. This finding is in agreement with previous studies (Wolever and Bolognesi 1996a). The difference between capillary and venous blood levels is most likely due to the uptake of glucose by forearm muscles when blood flows from the fingertip capillary to the vein in the antecubital fossa. Thus, the greatest difference between capillary and venous blood is seen postprandially. Moreover, the largest difference has been found in healthy subjects who have enhanced peripheral insulin sensitivity, e.g. in young, lean, and fit athletes, and the smallest difference in insulin-resistant subjects (Coppack et al. 1990, Jackson et al. 1983, Marks 1996). Our subjects were lean and their glucose tolerance was screened by OGTT as normal before study enrolment. However, insulin sensitivity was not measured.

Capillary blood sampling is preferred for determining GI values, but it is also acceptable to use venous blood sampling (FAO/WHO 1998). We found that the GIs based on capillary blood sampling were lower than those based on venous blood sampling, which is in accordance with the data from Granfeldt et al. (1995).



Similarly to earlier findings (Wolever et al. 2003), we observed that venous blood sampling elicited markedly higher inter-individual variation in GI values (exception rye bread when glucose solution was used as the reference food) than capillary blood sampling. This suggests that capillary blood may allow smaller differences to be detected (Granfeldt et al. 1995, Wolever and Bolognesi 1996b, Vrolix and Mensink 2010). Thus, fingertip capillary blood has been recommended in GI testing because of the greater sensitivity (Brouns et al. 2005, ISO 26642:2010. 2010). Our results confirm the superiority of capillary sampling.

According to the recommendation of FAO/WHO, GI measurements should be based on collection of seven blood samples at 15- to 30-min intervals over 2 h (FAO/WHO 1998), but the effect of frequency of blood sampling has not been widely studied (Wolever 2004). We found that the GI values calculated by using only four time-points (0, 30, 60 and 120 min) were 3% higher for oatmeal porridge ( $76 \pm 23$  vs.  $78 \pm 30$ ) and 9% higher for rye bread ( $78 \pm 33$  vs.  $86 \pm 40$ ), but were not higher for mashed potato ( $79 \pm 22$  vs.  $79 \pm 28$ ). The disparity between rye bread and oat porridge could be explained by lower blood glucose concentration at the 15-min time-point and higher blood glucose concentration at the 45- and 90-min time-points after eating rye bread. In other words, rye bread initially increases and then after the peak value is attained decreases the blood glucose more gradually than oat porridge. Reducing the frequency of blood sampling also produced higher standard deviation and larger variation of GI for all test foods. Our results are in line with an earlier study that demonstrated ~5% increase in GI values and higher SDs and larger variation when sampling frequency was reduced and was based on 4-5 time-points (Wolever 2004). When we extended blood sampling with an additional blood sample taken at 180 min, the GI values were virtually identical for oatmeal porridge ( $76 \pm 23$  vs.  $77 \pm 25$ ) and mashed potato ( $79 \pm 22$  vs.  $79 \pm 24$ ), but not for rye bread ( $78 \pm 33$  vs.  $84 \pm 42$ ). This finding highlights the fact that rye bread produces more stable blood glucose levels which do not return to baseline values at the 120-min time-point.

Capillary blood samples are easier to obtain than venous blood samples, and thus, the sampling requires less skill. In addition, capillary sampling is less invasive than the insertion of a cannula in the forearm. When several venous samples must be collected, a cannula inserted into a vein in the antecubital fossa could be more practical, but requires more qualified personnel. Haemolysis of red blood cells releases insulin-degrading enzyme, which degrades insulin and, as a consequence, reduces insulin concentration in the blood sample (Chevenne et al. 1998). Because of strong haemolysis, we had to exclude some insulin samples from the analysis. To avoid haemolysis, the subjects were advised to warm their hands under running warm water and nurses were advised not to squeeze the fingertip because squeezing may dilute samples with plasma, which decreases both glucose and insulin concentration and may rupture red blood cells. Clear and detailed instructions and a

practise session on how to take capillary blood for insulin analysis before starting the study are crucial.

#### **6.1.4 Reference food**

Both glucose solution and white bread can be used as a reference food (FAO/WHO 1998), but several other foods have also been used (Atkinson et al. 2008, Brouns et al. 2005). Originally, glucose solution was used as the reference in GI testing (Jenkins et al. 1981), but because of concerns that the osmotic effects of glucose solution could lead to delayed gastric emptying, white bread started to be used as a reference (Jenkins et al. 1983). The use of white bread is also advocated because it represents a more physiological meal than glucose solution (Wolever et al. 1991).

In the second interlaboratory study, subjects with a low average glucose IAUC of the reference food tended to have higher variation in reference food IAUC (Wolever et al. 2008). We did not find any correlation between the glucose solution reference IAUCs or the white bread reference IAUCs and within-person variability (data not shown). However, white bread as the reference produced smaller average IAUC with smaller within-subject variation.

We found that glucose produced 20-25% larger IAUCs than white bread when the reference test was repeated two or three times. However, the variation was slightly smaller when white bread was used as the reference. The mean CV of the capillary IAUCs for glucose solution was 36% and for white bread 28%, but different results have also been reported (Wolever et al. 1996, Wolever et al. 2003). Our results are in agreement with previous findings suggesting that a standardized starchy meal could allow more precise definition of 2-h postprandial glycaemia (Wolever et al. 1996).

White bread and its composition can, however, vary notably. As in our study, the amounts of available carbohydrates should be measured instead of taking the amount from local food tables (Wolever et al. 2003). We also used the same frozen and defrosted white bread throughout the study. Glucose response has been demonstrated to be significantly different between fresh, frozen, and defrosted white bread, possible due to an increased amount of retrograded starch during freezing (Burton and Lightowler 2008). This indicates the superiority of glucose solution as the reference. Thus, for international standardization, it is more reliable to use glucose solution as the reference food (Brouns et al. 2005, Wolever et al. 2003).

#### **6.1.5 Number of reference food tests**

To reduce within-subject variation of blood glucose, the recommendation is that the reference food should be tested at least three times (FAO/WHO 1998, Wolever et al. 1991), but a literature search revealed that the recommendation has not been systemically followed (Atkinson et al. 2008). We determined the effect of using

only a single test of reference food on calculating the GI values compared with using two or three tests. We found that testing the reference food twice essentially diminished the variation. The variation in our study was slightly higher, 35-36% for the glucose solution and 28% for white bread, when capillary blood sampling was used, but when we calculated the absolute percentage of difference, it was slightly lower for glucose solution. Wolever et al. (2003) examined how repeating the reference test influenced GI values. They compared three tests with one test and found that when three reference tests were used both the measured GI value and its variation were lower. As known, the distribution of GI values in individual subjects is skewed to a higher value, thus, repeating IAUCs will reduce skewness (Wolever et al. 2008). Increasing the replicate number of the reference food test to three or four clearly decreased variation (Vega-Lopez et al. 2007, Williams et al. 2008).

In Study I, we also found that the variation considerably decreased when the test food (white bread) was tested two or three times. However, GI values of white bread still varied widely between individuals. Our observations are in agreement with earlier findings (Vega-Lopez et al. 2007).

### 6.1.6 Coffee

Based on our study, coffee does not modify postprandial glycaemic and insulinaemic responses induced by carbohydrates. We examined the postprandial responses to two different portions of coffee ingested with a glucose solution. In the large coffee portion, the caffeine content was twofold that of the small portion. Despite the twofold difference in caffeine content, both portions induced an equal glucose response that was similar to a pure glucose solution ingested with water. Our results are in line with previous findings suggesting that coffee does not significantly affect 2-h postprandial glucose responses in healthy subjects (Aldughpassi and Wolever 2009a, Battram et al. 2006, Johnston et al. 2003, Pizziol et al. 1998, Young and Wolever 1998). Contradictory findings have also been reported. Caffeinated coffee compared with decaffeinated coffee significantly enhanced postprandial glycaemia over a 2-h period therefore also increasing the insulin responses in both healthy subjects (Moisey et al. 2008) and subjects with type 2 diabetes (Lane et al. 2007) after mixed-meal tolerance tests (MMTTs).

Earlier studies performed with caffeine capsules or caffeine infusion have shown a decreased insulin sensitivity in healthy humans (Battram et al. 2006, Keijzers et al. 2002, Thong and Graham 2002), in individuals with type 2 diabetes (Lane et al. 2004, Robinson et al. 2004), and in obese men (Petrie et al. 2004). Our findings, however, suggest that caffeine in coffee does not significantly alter postprandial insulin response. A previous study comparing the effects of caffeine capsules (4.45 mg/kg) and coffee with the same caffeine amount on insulin responses during an OGTT found almost 20% lower insulin response after drinking coffee than after

ingesting caffeine capsules (Battram et al. 2006). These findings imply that other components of coffee may improve glucose metabolism or attenuate the negative effect of caffeine. Chlorogenic acid found in coffee have been demonstrated to delay intestinal absorption (Johnston et al. 2003, van Dijk et al. 2009). However, when we estimated the impact of coffee on absorption by measuring the IAUcs 0 to 30 min (data not shown), the two different portion sizes of coffee yielded similar glucose responses. Thus, it is obvious that GI values are mainly determined by the carbohydrate source, and only a moderate impact of coffee on measured GI values existed. However, coffee diminished the variation, which is consistent with previous research (Aldughpassi and Wolever 2009b, Wolever et al. 2008).

## 6.2 Subjects' characteristics

In our study, overweight subjects had 20% larger 2-h glucose response than normal-weight subjects. However, subjects' BMI did not affect GI values, consistent with previous studies (Wolever et al. 1998a, Wolever et al. 2008, Wolever et al. 2009). Glucose response also increased significantly when subjects had impaired glucose tolerance (IGT) compared with healthy individuals, but subjects' glucose tolerance did not affect GI values. This finding is also in accordance with earlier results (Atkinson et al. 2008, Indar-Brown et al. 1992, Wolever et al. 1998b). IGT increased insulin response, but the difference was not significant relative to healthy subjects. When IGT and overweight manifested together, the insulin response increased significantly and was over twofold that of normal-weight subjects with normal glucose tolerance (NGT). Protein content of food has been shown to enhance insulin secretion in diabetic subjects (Nuttall et al. 1984, Simpson et al. 1985a). Our test meals contained 14 g of protein, which may partly explain the greater insulin response in subjects with IGT, but we did not observe significant differences in GIs. This finding is in line with previous studies (Wolever et al. 1998a). In insulin-sensitive subjects, ingested protein has enhanced the decline in blood glucose, but no relationship between insulin sensitivity and protein-stimulated insulinaemia has been demonstrated (Brand-Miller et al. 2000).

It is well-known that obesity is associated with insulin resistance (Eckel et al. 2011, Johnson and Olefsky 2014, Kahn et al. 2006, Olefsky et al. 1985). In our study, overweight clearly increased insulin responses regardless of the level of glucose tolerance. We found that the insulin response was over twofold in overweight subjects relative to normal-weight subjects. This finding is in line with previous studies that have established a higher postprandial insulin response in overweight subjects than in their normal-weight peers (Jensen et al. 1999, Ramel et al. 2009, Umpaichitra et al. 2004). When overweight and IGT manifested together, the highest insulin responses to the study meals and the reference food were found.

This phenomenon has also been seen in earlier studies (Simpson et al. 1985a, Wolever et al. 1998b).

Nevertheless, we found no significant differences in GI values in normal-weight subjects relative to overweight subjects. A recent study observed that BMI correlated negatively with GI values (Al Dhaheri et al. 2010). Consequently, new studies with a larger number of subjects are needed to determine the effect of BMI on GI values more comprehensively.

## 6.3 Macronutrients

Carbohydrate-rich foods are typically eaten as part of a mixed meal. It is a well-known that portion size, i.e. the larger amount of available carbohydrates, influences measured mean glycaemic responses (Wolever et al. 1996), which also affects the interpretation of the results. It is also known that both protein and fat decrease postprandial glycaemia and that protein enhances insulin secretion when ingested with a carbohydrate-rich meal (Gannon et al. 1993a). Carbohydrates has been suggested to explain about 90% of glycaemic response, with both protein and fat having only negligible effects (Wolever et al. 2006a). We found that adding a fat or a protein component either alone or together to a mashed potato-based meal decreased glycaemic responses. The GI values decreased considerably after the addition of oil and/or protein to the mashed potato meal, in line with earlier findings (Flint et al. 2004). Because GI testing is normally based on individual food items, much discussion has centred around measuring GI values for mixed meals and whether it is possible to predict the GI value of a meal (Dodd et al. 2011, Flint et al. 2005, Wolever et al. 2006a, Wolever and Bhaskaran 2012). It has also been suggested that GI values should not be measured for mixed meals (Wolever 2013a).

### 6.3.1 Fat

Earlier studies have found that fat ingested with potato lowers glycaemic responses (Collier and O'Dea 1983, Ercan et al. 1994, Gannon et al. 1993a). However, among subjects with type 2 diabetes no effect on maximal plasma glucose or mean glucose area has been observed (Gannon et al. 1993a). In addition, it has previously been shown that the different degrees of saturation of added fat did not affect glycaemic responses or GI values (MacIntosh et al. 2003). Contrarily, when healthy subjects co-ingested 15 g of sunflower oil with boiled potatoes, an elevated GI was obtained (Leeman et al. 2008). We observed that the addition of 30 g of rapeseed oil to a mashed potato meal reduced glycaemic responses, resulting in a 37-unit smaller GI value than for the consumption of mashed potato alone. This finding is in line with earlier studies (Dodd et al. 2011, Henry et al. 2006, MacIntosh et al. 2003).

As known, fat decreases glycaemic response when added to a carbohydrate-rich meal, but the effect is only seen with relatively large amounts of added fat. Moreover, the effect of fat on glycaemic responses is not linear (Normand et al. 2001, Owen and Wolever 2003). When the impact of varying fat across its normal range of intake (from 0 g to 40 g) was examined, it was found that 40 g of fat reduced glucose IAUC by 30% and 5 g of fat produced more than half of this effect. Thus, the glycaemic response elicited by white bread was reduced dose-dependently by adding fat, but the dose-response curve was not linear (Owen and Wolever 2003). A recent study assessed the dose-dependent effect of fat on GI values (with 5-30 g of corn oil). There was no significant effect of fat on GI values in healthy subjects (Moghaddam et al. 2006). Even when we found a 34% decrease in the GI value of mashed potato with 30 g of rapeseed oil, this decrease was not significant. A larger number of subjects may be needed to detect a significant difference in GI value with this amount of fat (Williams et al. 2008).

### 6.3.2 Protein

Adding a protein component to the mashed potato meal reduced glucose response, and insulin response. Our results are line with an earlier study with similar amounts of protein (25-50 g of protein added to 50 g of carbohydrate) showing that postprandial glucose response decreased due to increased insulin response (Gannon et al. 1988, Nuttall et al. 1984).

Protein is inversely associated with GI values and moreover has a 2- to 3-fold greater reducing gram-for-gram impact on glycaemic response than fat (Moghaddam et al. 2006). We found that the co-ingestion of fat and protein with mashed potato decreased glycaemic response and the GI values, consistent with an earlier study (Gulliford et al. 1989).

Our finding that the mashed potato-based meals produced high insulin responses is line with previous observations (Coulston et al. 1984a). As a consequence of accumulating data, potato has been classified as one of the most insulinogenic foods (Holt et al. 1997). We measured an II of 118 for mashed potato, which is consistent with the previously reported value of 128 for instant mashed potato (Lan-Pidhainy and Wolever 2011). In our study, the mixed meal containing the protein component evoked the largest insulin response, thus markedly increasing the II. However, adding fat to the meal attenuated the increasing effect of protein on insulinaemic responses. Previous studies have shown that ingested protein significantly increases insulinaemia (Bornet et al. 1987, Brand-Miller et al. 2000, Gannon et al. 1988, Gulliford et al. 1989, Krezowski et al. 1986, Nuttall et al. 1984, Nuttall and Gannon 1991), while fat reduces insulin responses (Gannon et al. 1993a, Gulliford et al. 1989, Welch et al. 1987), which is in line with our findings. Thus, the reduced glycaemia is clearly explained by the enhanced insulin secretion (Gentilcore et al.

2006, Ma et al. 2009). When rapeseed oil, chicken breast, and salad were included in a mashed potato meal, the insulin response was lower than for mashed potato alone. This suggests that oil and salad were able to overcome the strong increase in insulin response that was induced by the protein source alone. This finding may be explained by the higher energy content of the meal, which slows gastric emptying (Kwiatek et al. 2009). Possibly, the effect of fat on glycaemic responses may depend on insulin sensitivity. Moghaddam et al. (2006) found that fat reduced glycaemic responses less in hyperinsulinaemic subjects than in subjects with normal fasting insulin, but contradictory findings also exist.

A positive correlation between GI and II values emerged in several studies (Björck et al. 2000), but discrepant results have also been presented (Flint et al. 2004). However, adding fat and protein components to starchy meals significantly increased the IIs of the meals in type 2 diabetic subjects (Bornet et al. 1987). We found that protein enhanced insulin response and increased the IIs of carbohydrate-rich meals, and simultaneously, glucose responses and GI values diminished in healthy subjects. These findings are in accord with previous reports (Bao et al. 2009).

### 6.3.3 Alcohol

We found that alcohol increases postprandial glucose and insulin responses. This finding suggests that alcohol acutely impairs insulin sensitivity. Earlier studies have shown that alcohol induces hypoglycaemia in type 1 diabetic subjects, which is mainly mediated by the inhibition of gluconeogenesis and glycogenolysis (van de Wiel 2004). However, moderate amounts of alcohol seemed to enhance insulin secretion, while not notably affecting postprandial blood glucose in type 2 diabetic subjects (Christiansen et al. 1993). Acute alcohol consumption induces insulin resistance primarily in skeletal muscle (Ting and Lout 2006). A potential mechanism causing insulin resistance may be increased postprandial triacylglycerol responses, seen in both healthy subjects (Fielding et al. 2000, Raben et al. 2003) and those with type 2 diabetes (Dalgaard et al. 2004). Moreover, no changes in glucose and insulin levels were observed in response to alcohol in a hyperinsulinaemic, euglycaemic clamp study (Christiansen et al. 1996). In addition, a light meal has been proposed to eliminate the insulinogenic effect of moderate alcohol intake in type 2 diabetic subjects (Christiansen et al. 1994). There are, however, only a few studies focusing on the postprandial effect of alcohol in healthy subjects. The impact of alcohol on postprandial glucose and insulin responses has been inconsistent when alcohol is served with meals (Brand-Miller et al. 2007, Fielding et al. 2000, Greenfield et al. 2005, Suter et al. 2001).

Beer produced a very high GI value of 119 (i.e. >70), and non-alcoholic beer a high GI value of 80. The measured GI values are in line with the GI values

published the brewing industry, 101 to 120 for four different beers (Walker 2006). However, to our knowledge, only one GI value of 66 for beer has been published (Brand-Miller et al. 2007), but the value is based on a non-standard 10-g carbohydrate portion size. Less than 20 g of available carbohydrate has been found to produce very large within-subject variation of IAUC, which may result in an imprecise estimate of GI (Wolever et al. 2006b). In the literature, a glucose score of 58 has also been presented for beer. However, this score was measured with a 1000-kJ portion of beer containing 33 g of alcohol and 13 g of carbohydrates and using a white bread containing 44 g of carbohydrates as the reference (Brand-Miller et al. 2007). Because of the study design, the glucose score of beer is not comparable with the measured GI values of beer.

A major strength of our study was that the amount of available carbohydrate in the beer was based on laboratory analysis. The analysis showed that the available carbohydrates in beer were starch and malto-oligosaccharides. Earlier studies have revealed that starchy foods have high GI values (Brand-Miller et al. 2009), and the GI values from 75 to 105 for maltose have been reported (Jenkins et al. 1981, Yang et al. 2006). These findings support our result of non-alcoholic beer producing a high GI value of 80. Non-alcoholic beer tended to produce smaller postprandial glucose response than the reference glucose solution, most likely due to the presence of complex malto-oligosaccharides. Products of fermentation have been speculated to result in a pronounced inhibitory effect of gastric emptying relative to the corresponding pure alcohol concentrations (Franke et al. 2004). However, beer and alcohol in glucose solution gave similar responses, which may suggest that alcohol catalyses the breakdown of complex carbohydrates into glucose. This may explain the significantly larger glucose response of beer than of non-alcoholic beer.

Epidemiological studies focusing on the association between GI and chronic diseases have used highly variable GI values for beer, ranging from 36 to 95 (Flood et al. 2006, Neuhouwer et al. 2006, Schulz et al. 2005). Commonly, the imputed GI values have been based on other carbohydrate-rich beverages, such as milk and orange juice. Depending on the amount of consumed beer, the misclassification of beer into the group of low GI foods may cause substantial bias in epidemiological studies.



# 7 Conclusions

This thesis contributes to the information on the relationship between different methodological choices and measurement of GI values of carbohydrate-rich foods. Specific findings of each study were as follows:

## *Study I*

Capillary blood samples should be used when measuring GI values. In addition, the reference food should be tested at least twice and glucose solution should be used as a reference food to improve the accuracy of the measured GI value.

## *Study II*

Coffee as such does not modify the glucose and insulin responses to a carbohydrate food. Thus, coffee can be the beverage of choice in GI testing.

## *Study III*

Both overweight and impaired glucose tolerance increased glycaemic and insulinaemic responses to the tested meals and the reference food. As a consequence, subjects' physiological characteristics, body weight, and glucose tolerance do not affect the measured GI values.

## *Study IV*

Both fat and protein have an independent decreasing effect on glycaemia. A mashed potato-based meal including a high-fat or high-protein meal component induces a substantially lower glycaemic response than mashed potato alone.

## *Study V*

Alcohol increases postprandial glucose and insulin responses, probably through ethanol-induced insulin resistance. Beer has a high GI value that should be taken into account when GI databases are compiled for epidemiologic research.



## 8 Future perspectives

Recently, much discussion has centred around the validity of measured GI values and how useful the concept of GI is for the general public and for food labelling (Aziz et al. 2013, Chiu et al. 2011, Hare-Bruun et al. 2008, Wolever 2013a, Wolever 2013b). The GI values for different foods and food combinations have been measured for three decades. The methodological approaches to GI measurement remain under debate despite the recently published international standard (International Standard ISO 26642:2010).

The question arising from the findings of this thesis is how to overcome effects of methodological choices in GI testing. The concept of GI is highly complex. The physicochemical properties of carbohydrates and carbohydrate-rich foods, e.g. the chemical structure of carbohydrate, the food matrix, and food processing, have an impact on postprandial responses. The GI can be one quality measure of carbohydrate-rich foods, but should not to be the only one. The GI is a reliable tool for distinguishing between low- and high-GI foods, with some exceptions. It is possible that different population groups, e.g. groups of different ethnic backgrounds, may considerably affect the classification of the GI values.

Many open questions remain concerning the validity of measured GI values, e.g. questioning the number of study subjects needed and the background of subjects, despite the published international standard (ISO 26642:2010). Several earlier GI studies were conducted with insufficient numbers of study subjects (<10), but these values are still widely used in international databases of GI values, and, as a consequence, in scientific research. Is the GI a valid method for evaluating the quality of carbohydrates? The answer to this is not straightforward. Open-minded scientific discussion and more methodological research are warranted.

In GI testing, the number of subjects should be increased. Unfortunately, this means that many of the earlier GI studies need to be repeated with sufficient numbers of subjects. In addition, the 10 test subjects recommended in the international standard may still be too low. Open questions remain concerning the background of test subjects. Recent research has investigated ethnicity and how it affects postprandial glycaemia and insulinaemia. It has also been suggested that the II is clinically less useful because it varies in different groups of subjects, but thus far only a few studies have assessed the differences between groups of different physiological backgrounds. In addition, GI measurements are often confounded by other food components, such as fat and protein, and, people with different degrees of insulin sensitivity are known to react differently to the protein content of food (Brand-Miller et al. 2000).

Measured insulin responses are required to provide physiological explanations for different glycaemic responses to similar foods, e.g. breads. Recent interest has

focused on factors other than fat or protein that can modify postprandial responses to different type of breads such as white and rye bread. It has constantly been shown that rye bread produce lower insulin response than white bread despite similar GI values (Juntunen et al. 2002, Juntunen et al. 2003a, Leinonen et al. 1999, Törrönen et al. 2013). This highlights the fact that there is an urgent need for insulin measurements of carbohydrate-rich foods to clarify which starchy foods with similar GI values are better nutritional choices in the same food group. In other words, insulin responses to carbohydrate-rich foods and their IIs are an important topic of for further studies. If valid measurements for both GIs and IIs become available, it would be easier to compile new research settings where the impact of quality of carbohydrates on health can be measured without confounding factors.

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